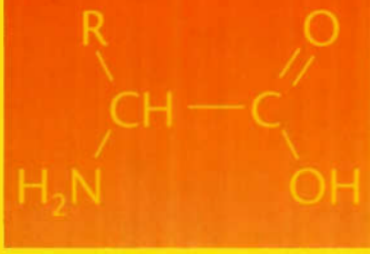
SPECIALIST PERIODICAL REPORTS

RS•C



Amino acids, *peptides* and *proteins*

Volume 33

senior reporters
G.C. BARRETT *and* J.S. DAVIES

Amino Acids, Peptides and Proteins

Volume 33

A Specialist Periodical Report

Amino Acids, Peptides and Proteins

Volume 33

A Review of the Literature Published during 2000

Senior Reporters
G.C. Barrett, *Oxford, UK*
J.S. Davies, *University of Wales, Swansea, UK*

Reporters
W. Chan, *University of Nottingham, UK*
D.T. Elmore, *University of Oxford, UK*
E. Farkas, *Lajos Kossuth University, Debrecen, Hungary*
A. Higton, *University of Nottingham, UK*
I. Sóvágó, *Lajos Kossuth University, Debrecen, Hungary*

RS•C
ROYAL SOCIETY OF CHEMISTRY

NEW FROM 2003

If you buy this title on standing order, you will be given FREE access to the chapters online. Please contact cockheadg@rsc.org with proof of purchase to arrange access to be set up.

Thank you.

ISBN 0-85404-237-7

ISSN 1361-5904

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at www.rsc.org

Typeset by Vision Typesetting, Manchester, UK
Printed by Athenaeum Press Ltd, Gateshead, Tyne and Wear, UK

Preface

This volume of reports, covering the literature of the year 2000, has seen some evolutionary changes to chapters, especially those that have been 'penned' by new or different authors compared with previous volumes. For continuity, authors have again been asked, as far as possible, to structure sub-sections to correlate with patterns used over a number of years. However, the depth of discussion has certainly been improved in many sections. The volume of international literature in this subject area continues to expand, with its thrust moving seamlessly into the more biological/medicinal areas.

Two Senior Reporters have overseen the compilation of this volume, which reflects the increased challenges in securing authors to undertake the demanding tasks of reviewing. Whereas the original literature has become available increasingly in electronic form, access to this form and to hard copy of journals is not always easy, and hence the task of the reviewer seems to have become harder rather than easier. The policy adopted by each reporter is to concentrate on refereed papers, with little coverage of conference proceedings and patents.

This volume see the inclusion of the biennial chapter on 'Metal Complexes of Amino Acids and Peptides' written by our colleagues from Hungary (Etelka Farkas and Imre Sóvágó), while Donald Elmore has maintained his comprehensive coverage of Peptide Synthesis. Graham Barrett has taken a more in-depth approach to the chapter on Proteins, which is reflected by a change in title for the chapter. Having the same authorship (John Davies) for the middle chapters in this volume has meant that the tendency for cyclic peptides to achieve the honour of being reviewed in both chapters has now been virtually eliminated. We welcome Weng Chan and Avril Higton as new authors for the Amino Acids review, hoping that having 'cut their teeth' in this field, they will wish to continue to contribute to this Specialist Periodical Report.

Finally, as deadlines come and go, we are perennially grateful to the RSC staff for their patience and efficient transfer of the reporters' hard work into electronic and printed compilations, and for adopting the use of figures in colour for the electronic version of the chapter on Proteins. We sincerely hope that the wealth of material reviewed here will inspire our readers to contribute further key developments to this area of great endeavour.

Graham Barrett
Oxford

John Davies
Swansea

Contents

Amino Acids, Peptides and Proteins, Volume 33

A Short Guide to Abbreviations and Their Use in Peptide Science

Abbreviations, acronyms and symbolic representations are very much part of the language of peptide science – in conversational communication as much as in its literature. They are not only a convenience, either – they enable the necessary but distracting complexities of long chemical names and technical terms to be pushed into the background so the wood can be seen among the trees. Many of the abbreviations in use are so much in currency that they need no explanation. The main purpose of this editorial is to identify them and free authors from the hitherto tiresome requirement to define them in every paper. Those in the tables that follow – which will be updated from time to time – may in future be used in this Journal without explanation.

All other abbreviations should be defined. Previously published usage should be followed unless it is manifestly clumsy or inappropriate. Where it is necessary to devise new abbreviations and symbols, the general principles behind established examples should be followed. Thus, new amino-acid symbols should be of form Abc, with due thought for possible ambiguities (Dap might be obvious for diaminoproprionic acid, for example, but what about diaminopimelic acid?).

Where alternatives are indicated below, the first is preferred.

Amino Acids

Proteinogenic Amino Acids

Ala	Alanine	A
Arg	Arginine	R
Asn	Asparagine	N
Asp	Aspartic acid	D
Asx	Asn *or* Asp	
Cys	Cysteine	C
Gln	Glutamine	Q
Glu	Glutamic acid	E
Glx	Gln *or* Glu	
Gly	Glycine	G
His	Histidine	H
Ile	Isoleucine	I
Leu	Leucine	L
Lys	Lysine	K

Met	Methionine	M
Phe	Phenylalanine	F
Pro	Proline	P
Ser	Serine	S
Thr	Threonine	T
Trp	Tryptophan	W
Tyr	Tyrosine	Y
Val	Valine	V

Other Amino Acids

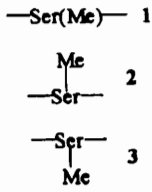
Aad	α-Aminoadipic acid
βAad	β-Aminoadipic acid
Abu	α-Aminobutyric acid
Aib	α-Aminoisobutyric acid; α-methylalanine
βAla	β-Alanine; 3-aminopropionic acid (avoid Bal)
Asu	α-Aminosuberic acid
Aze	Azetidine-2-carboxylic acid
Cha	β-cyclohexylalanine
Cit	Citrulline; 2-amino-5-ureidovaleric acid
Dha	Dehydroalanine (also ΔAla)
Gla	γ-Carboxyglutamic acid
Glp	pyroglutamic acid; 5-oxoproline (also pGlu)
Hph	Homophenylalanine (Hse = homoserine, and so on). Caution is necessary over the use of the use of the prefix homo in relation to α-amino-acid names and the symbols for homo-analogues. When the term first became current, it was applied to analogues in which a side-chain CH_2 extension had been introduced. Thus homoserine has a side-chain CH_2CH_2OH, homoarginine $CH_2CH_2CH_2NHC(=NH)NH_2$, and so on. In such cases, the convention is that a new three-letter symbol for the analogue is derived from the parent, by taking H for homo and combining it with the first two characters of the parental symbol – hence, Hse, Har and so on. Now, however, there is a considerable literature on β-amino acids which are analogues of α-amino acids in which a CH_2 group has been inserted between the α-carbon and carboxyl group. These analogues have also been called homo-analogues, and there are instances for example not only of 'homophenylalanine', $NH_2CH(CH_2CH_2Ph)CO_2H$, abbreviated Hph, but also 'homophenylalanine', $NH_2CH(CH_2Ph)CH_2CO_2H$ abbreviated Hph. Further, members of the analogue class with CH_2 interpolated between the α-carbon and the carboxyl group of the parent α-amino acid structure have been called both 'α-homo'- and 'β-homo'. Clearly great care is essential, and abbreviations for 'homo' analogues ought to be fully defined on every occasion. The term 'β-homo' seems preferable for backbone extension (emphasizing as it does that the residue has become a β-amino acid residue), with abbreviated sym-

	bolism as illustrated by βHph for $NH_2CH(CH_2Ph)CH_2CO_2H$.
Hyl	δ-Hydroxylysine
Hyp	4-Hydroxyproline
αIle	*allo*-Isoleucine; 2*S*, 3*R* in the L-series
Lan	Lanthionine; *S*-(2-amino-2-carboxyethyl)cysteine
MeAla	*N*-Methylalanine (MeVal = *N*-methylvaline, and so on). This style should not be used for α-methyl residues, for which either a separate unique symbol (such as Aib for α-methylalanine) should be used, or the position of the methyl group should be made explicit as in αMeTyr for α-methyltyrosine.
Nle	Norleucine; α-aminocaproic acid
Orn	Ornithine; 2,5-diaminopentanoic acid
Phg	Phenylglycine; 2-aminophenylacetic acid
Pip	Pipecolic acid; piperidine-s-carboxylic acid
Sar	Sarcosine; *N*-methylglycine
Sta	Statine; (3*S*, 4*S*)-4-amino-3-hydroxy-6-methyl-heptanoic acid
Thi	β-Thienylalanine
Tic	1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid
αThr	*allo*-Threonine; 2*S*, 3*S* in the L-series
Thz	Thiazolidine-4-carboxylic acid, thiaproline
Xaa	Unknown or unspecified (also Aaa)

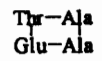
The three-letter symbols should be used in accord with the IUPAC-IUB conventions, which have been published in many places (e.g. *European J. Biochem.* 1984; **138**: 9–37), and which are (May 1999) also available with other relevant documents at: http://www.chem.qnw.ac.uk/iubmb/iubmb.html#03

It would be superfluous to attempt to repeat all the detail which can be found at the above address, and the ramifications are extensive, but a few remarks focussing on common misuses and confusions may assist. The three-letter symbol standing alone represents the unmodified intact amino acid, of the L-configuration unless otherwise stated (but the L-configuration may be indicated if desired for emphasis: e.g. L-Ala). The same three-letter symbol, however, also stands for the corresponding amino acid *residue*. The symbols can thus be used to represent peptides (e.g. AlaAla or Ala-Ala = alanylalanine). When nothing is shown attached to either side of the three-letter symbol it is meant to be understood that the amino group (always understood to be on the left) or carboxyl group is unmodified, but this can be emphasized, so AlaAla = H-AlaAla-OH. Note however that indicating free termini by presenting the terminal group in full is wrong; $NH_2AlaAlaCO_2H$ implies a hydrazino group at one end and an α-keto acid derivative at the other. Representation of a free terminal carboxyl group by writing H on the right is also wrong because that implies a terminal aldehyde.

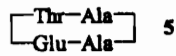
Side chains are understood to be unsubstituted if nothing is shown, but a substituent can be indicated by use of brackets or attachment by a vertical bond up or down. Thus an *O*-methylserine residue could be shown as **1**, **2**, or **3**.



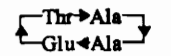
Note that the oxygen atom is not shown: it is contained in the three-letter symbol – showing it, as in Ser(OMe), would imply that a peroxy group was present. Bonds up or down should be used only for indicating side-chain substitution. Confusions may creep in if the three-letter symbols are used thoughtlessly in representations of cyclic peptides. Consider by way of example the hypothetical cyclopeptide threonylalanylalanylglutamic acid. It might be thought that this compound could be economically represented **4**.



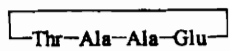
But this is wrong because the left hand vertical bond implies an ester link between the two side chains, and strictly speaking if the right hand vertical bond means anything it means that the two Ala α-carbons are linked by a CH_2CH_2 bridge. This objection could be circumvented by writing the structure as in **5**.



But this is now ambiguous because the convention that the symbols are to be read as having the amino nitrogen to the left cannot be imposed on both lines. The direction of the peptide bond needs to be shown with an arrow pointing from CO to N, as in **6**.



Actually the simplest representation is on one line, as in **7**.



Substituents and Protecting Groups

Ac	Acetyl
Acm	Acetamidomethyl
Adoc	1-Adamantyloxycarbonyl
Alloc	Allyloxycarbonyl
Boc	*t*-Butoxycarbonyl
Bom	π-Benzyloxymethyl
Bpoc	2-(4-Biphenylyl)isopropoxycarbonyl

Btm	Benzylthiomethyl
Bum	π-*t*-Butoxymethyl
Bu^i	*i*-Butyl
Bu^n	*n*-Butyl
Bu^t	*t*-Butyl
Bz	Benzoyl
Bzl	Benzyl (also Bn); Bzl(OMe) = 4-methoxybenzyl and so on
Cha	Cyclohexylammonium salt
Clt	2-Chlorotrityl
Dcha	Dicyclohexylammonium salt
Dde	1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl
Ddz	2-(3,5-Dimethoxyphenyl)-isopropoxycarbonyl
Dnp	2,4-Dinitrophenyl
Dpp	Diphenylphosphinyl
Et	Ethyl
Fmoc	9-Fluorenylmethoxycarbonyl
For	Formyl
Mbh	4,4′-Dimethoxydiphenylmethyl, 4,4′-Dimethoxybenzhydryl
Mbs	4-Methoxybenzenesulphonyl
Me	Methyl
Mob	4-Methoxybenzyl
Mtr	2,3,6-Trimethyl,4-methoxybenzenesulphonyl
Nps	2-Nitrophenylsulphenyl
OAll	Allyl ester
OBt	1-Benzotriazolyl ester
OcHx	Cyclohexyl ester
ONp	4-Nitrophenyl ester
OPcp	Pentachlorophenyl ester
OPfp	Pentafluorophenyl ester
OSu	Succinimido ester
OTce	2,2,2-Trichloroethyl ester
OTcp	2,4,5-Trichlorophenyl ester
Tmob	2,4,5-Trimethoxybenzyl
Mtt	4-Methyltrityl
Pac	Phenacyl, $PhCOCH_2$ (care! Pac also = $PhCH_2CO$)
Ph	Phenyl
Pht	Phthaloyl
Scm	Methoxycarbonylsulphenyl
Pmc	2,2,5,7,8-Pentamethylchroman-6-sulphonyl
Pr^i	*i*-Propyl
Pr^n	*n*-Propyl
Tfa	Trifluoroacetyl
Tos	4-Toluenesulphonyl (also Ts)
Troc	2,2,2-Trichloroethoxycarbonyl
Trt	Trityl, triphenylmethyl
Xan	9-Xanthydryl

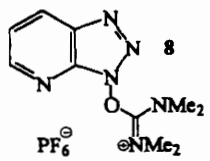
Z Benzyloxycarbonyl (also Cbz). Z(2Cl) = 2-chlorobenzyloxycarbonyl and so on

Amino Acid Derivatives

DKP Diketopiperazine
NCA *N*-Carboxyanhydride
PTH Phenylthiohydantoin
UNCA Urethane *N*-carboxyanhydride

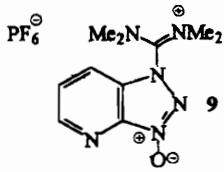
Reagents and Solvents

BOP 1-Benzotriazolyloxy-tris-dimethylamino-phosphonium hexafluorophosphate
CDI Carbonyldiimidazole
DBU Diazabicyclo[5.4.0]-undec-7-ene
DCCI Dicyclohexylcarbodiimide (also DCC)
DCHU Dicyclohexylurea (also DCU)
DCM Dichloromethane
DEAD Diethyl azodicarboxylate (DMAD = the dimethyl analogue)
DIPCI Diisopropylcarbodiimide (also DIC)
DIPEA Diisopropylethylamine (also DIEA)
DMA Dimethylacetamide
DMAP 4-Dimethylaminopyridine
DMF Dimethylformamide
DMS Dimethylsulphide
DMSO Dimethylsulphoxide
DPAA Diphenylphosphoryl azide
EEDQ 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
HATU This is the acronym for the 'uronium' coupling reagent derived from HOAt, which was originally thought to have the structure **8**, the ***H***exafluorophosphate salt of the *O*-(7-***A***zabenzotriazol-lyl)-***T***etramethyl ***U***ronium cation.



8

In fact this reagent has the isomeric *N*-oxide structure **9** in the crystalline state, the unwieldy correct name of which does not conform logically with the acronym, but the acronym continues in use.



9

	Similarly, the corresponding reagent derived from HOBt has the firmly attached label HBTU (the tetrafluoroborate salt is also used: TBTU), despite the fact that it is not actually a uronium salt.
HMP	Hexamethylphosphoric triamide (also HMPA, HMPTA)
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
HOCt	1-Hydroxy-4-ethoxycarbonyl-1,2,3-triazole
NDMBA	*N*,*N*′-Dimethylbarbituric acid
NMM	*N*-Methylmorpholine
PAM	Phenylacetamidomethyl resin
PEG	Polyethylene glycol
PtBOP	1-Benzotriazolyloxy-tris-pyrrolidinophosphonium hexafluorophosphate
SDS	Sodium dodecyl sulphate
TBAF	Tetrabutylammonium fluoride
TBTU	See remarks under HATU above
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFE	Trifluoroethanol
TFMSA	Trifluoromethanesulphonic acid
THF	Tetrahydrofuran
WSCI	Water soluble carbodiimide: 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide hydrochloride (also EDC)

Techniques

CD	Circular dichroism
COSY	Correlated spectroscopy
CZE	Capillary zone electrophoresis
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
ESR	Electron spin resonance
FAB	Fast atom bombardment
FT	Fourier transform
GLC	Gas liquid chromatography
hplc	High performance liquid chromatography
IR	Infra red
MALDI	Matrix-assisted laser desorption ionization
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
nOe	Nuclear Overhauser effect
NOESY	Nuclear Overhauser enhanced spectroscopy
ORD	Optical rotatory dispersion
PAGE	Polyacrylamide gel electrophoresis
RIA	Radioimmunoassay
ROESY	Rotating frame nuclear Overhauser enhanced spectroscopy
RP	Reversed phase

SPPS	Solid phase peptide synthesis
TLC	Thin layer chromatography
TOCSY	Total correlation spectroscopy
TOF	Time of flight
UV	Ultraviolet

Miscellaneous

Ab	Antibody
ACE	Angiotensin-converting enzyme
ACTH	Adrenocorticotropic hormone
Ag	Antigen
AIDS	Acquired immunodeficiency syndrome
ANP	Atrial natriuretic polypeptide
ATP	Adenosine triphosphate
BK	Bradykinin
BSA	Bovine serum albumin
CCK	Cholecystokinin
DNA	Deoxyribonucleic acid
FSH	Follicle stimulating hormone
GH	Growth hormone
HIV	Human immunodeficiency virus
LHRH	Luteinizing hormone releasing hormone
MAP	Multiple antigen peptide
NPY	Neuropeptide Y
OT	Oxytocin
PTH	Parathyroid hormone
QSAR	Quantitative structure–activity relationship
RNA	Ribonucleic acid
TASP	Template-assembled synthetic protein
TRH	Thyrotropin releasing hormone
VIP	Vasoactive intestinal peptide
VP	Vasopressin

J. H. Jones

1
Amino Acids

BY WENG C. CHAN and AVRIL HIGTON

1 Introduction

This chapter covers the newly published chemistry of amino acids for the year 2000; some biological aspects are also included to accompany the relevant chemical studies. Some references from 1999 stray into the list as do a few from early 2001. Literature citations were selected through Chemical Abstracts (Volume 132, Issue 6 to Volume 133, Issue 8) and through major journals, which frequently contain amino acid-related papers. Papers on related material have again been grouped without comment. Conference proceedings are largely excluded, and no patent material has been included.

Amino acid analogues containing oxy acids of phosphorus, and boron and sulfonic acids have been included in the section relevant to the type of amino acid considered, as have the increasing numbers of metal complexes containing amino acid ligands. References have been included in the section relevant to the primary theme of the paper, even if other aspects are also included.

2 Textbooks and Reviews

The reviews listed here only relate to general amino acid topics, specific reviews are mentioned in the appropriate section. Textbooks have appeared describing a practical approach to amino acid derivatives,[1] to amino acids as natural products,[2] and to phosphorus analogues of amino acids.[3]

Total syntheses of amino acids to the year 1998 have been reviewed.[4] Reviews of the syntheses of amino acids using biochemical, biosynthetic or a combination of chemical and biotechnological methods have been reported; the synthesis of amino acids by a combination of chemical and biochemical processes[5] and biotechnology[6] and biocatalytic production of atypical amino acids.[7] Engineering microbial pathways for amino acid production have been reviewed.[8] The stereoselective syntheses of amino acids using hydantoinases and carbamoylases have been reviewed,[9] as have new routes to chiral amino acids using biosynthetic pathways.[10] Reviews of asymmetric syntheses that do not fit comfortably into other sections have included; asymmetric synthesis of unnatural amino acids using commercially available chiral nonracemic glycinates[11] and the asymmetric

Amino Acids, Peptides and Proteins, Volume 33

synthesis of α-amino acids and β-amino acids using chiral zirconium complexes as catalysts.[12] The thermodynamics of the asymmetric synthesis of amino acids have also been reviewed.[13] Reviews have also appeared on the progress of the commercial synthesis of amino acids and other nutraceuticals,[14] on approaches to the total synthesis of natural product-based compound libraries using polymeric supports,[15] on tracer amino acids for the investigation of protein and amino acid metabolism in humans,[16] of the synthesis of the unusual amino acids found in peptides of aquatic origin and their incorporation into the peptides,[17] and on the uses of β-amino acids in medicinal chemistry and as building blocks for peptide modification have been reviewed.[18]

The etymology of amino acid names has been reviewed.[19]

3 Naturally Occurring Amino Acids

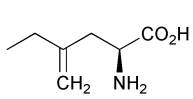
Studies have been reported on the composition and abundance of amino acids in ores from the supergene zones of Mingshan gold ore deposit,[20] and in the hydrothermally altered sediments from the Juan de Fuca ridge in the pacific ocean. Both the free and hydrolysable amino acid composition was analysed.[21] Naturally occurring aminophosphonic acids have been reviewed.[22]

3.1 Occurrences of Known Amino Acids. – Glutamic acid, glutamine, pyroglutamic acid and arginine have been isolated from the pronotal and elytral secretions of *Platyphora opima* and *Desmogramma subtropica* along with triterpene saponins and phosphatidylcholines.[23]

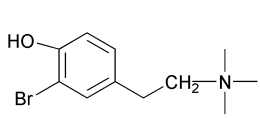
The amino acid *N′*-[(*R*)-1-carboxyethyl]-N^{α}-(D-galacturonyl)-L-lysine has been identified as a component of the O-specific polysaccharide of *Proteus mirabilis*.[24]

3.2 New Naturally Occurring Amino Acids. – (*S*)-2-Methylglutamine and (*S*)-5-methylarginine have been identified in the active site region of methyl-coenzyme M reductase. The biosynthesis of these and other methylated amino acids is discussed, together with the implication for the production of methane greenhouse gas.[25]

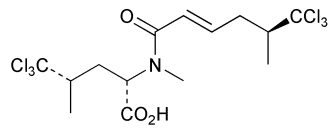
Sponges continue to be fertile ground for the discovery of novel amino acids; three new *N*-acyl-2-methylene-β-alanine methyl esters, hurghamids E–G, have been isolated from *Hippospongia* spp.[26] The amino acid (**1**) has been isolated from the Caribbean sponge *Plaktoris* simplex along with plakortones and simplactones.[27] The bromotyrosine compound (**2**) was isolated as a secondary metabolite from the sponge *Verulonga gigantea* and identified from spectral data,[28] and herbacic acid (**3**) has been isolated from the sponge *Dysidea herbacea*.[29] Synthetic studies have also been carried out.[30] The structure of pulcherrimine, a bitter-tasting amino acid from the sea urchin *Hemicentrotus pulcherrimus* was elucidated as (**4**) from chemical and spectral data.[31] Makalika ester and makalikone ester (**5**, X = CH_2 or CO, respectively) were isolated from the sea hare and their structures determined from spectral data.[32]



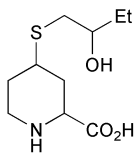
(1)



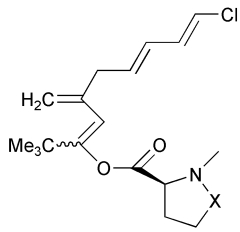
(2)



(3)



(4)



(5)

3.3 New Amino Acids from Hydrolysates. – The structures of microscleridermins F–I, isolated from the sponge *Microscleroderma* sp., were elucidated from chemical and spectral data. The compounds incorporate an unusual long chain β-amino acid.[33]

4 Chemical Synthesis and Resolution of Amino Acids

Readers seeking syntheses of particular amino acids should consult both Sections 4 and 6.3 of this chapter.

Two reviews on the synthesis of conformationally constrained aromatic amino acids are available.[34,35] Reviews of the preparation of aziridine carboxylates, carboxamides and lactones and their transformation into α- and β-amino acids,[36] and the synthesis of vinyl amino acids[37] have been published. A novel synthetic protocol for enantiomerically pure substituted prolines[38] has been reported. The synthesis of unnatural amino acids by reduction and ozonolysis of aromatic amino acids has been reviewed,[39] and on the synthesis of non-natural α-amino acid derivatives.[40] Recent advances in the synthesis and application of labelled nucleic acids, amino acids and carbohydrates,[41] and a review with eighteen references, including the preparation of Schiff bases, tandem reduction of and alkylation of Schiff base esters and the synthesis of complex amino acid polyols,[42] have been published.

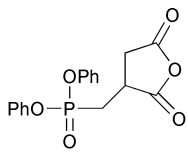
4.1 General Methods for the Synthesis of α-Amino Acids, Including Enantioselective Synthesis. – Recent synthetic advances in the preparation of phosphorus analogues of α-amino acids[43] and a rapid solid phase synthesis of non-proteinogenic *N*-acetyl α-amino acids[44] have been reported. A review (with forty-one references) examining practical catalysts and processes for the synthesis of both L- and D-amino acids using ligand systems derived from D-glucose has been published.[45]

The enthalpy of formation of peptides compared with values for the parent

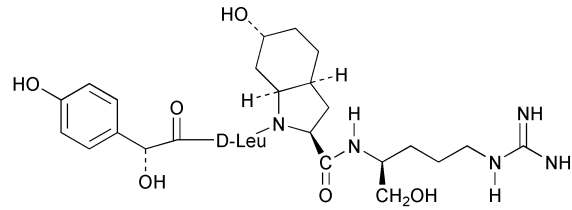
amino acids has been reviewed. The review also covers values for amides compared to carboxylic acids.[46]

Synthetic routes for the preparation of the model compound *e.g.* (**6**), an analogue of phosphoryl amino acids,[47] α-amino alkanephosphonic acids[48] and ω-aminophosphonic acids[49] have been described. The syntheses of compounds with side chain C–P links[50] and asymmetric synthesis[51] have been reviewed. A review of pentacoordinated phosphorus compounds of amino acids and nucleosides has been published.[52]

A procedure for the synthesis of (*R*)- and (*S*)-enantiomers of α-carbon deuterium-labelled α-amino acids has been described. The labelled enantiomers were resolved on a chiral ion exchanger.[53] The configuration of Aeruginosin 298-A (**7**) has been reassigned based on its total synthesis, incorporation of D-leucine gave the natural product.[54]



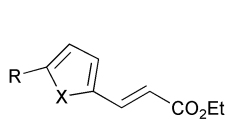
(6)



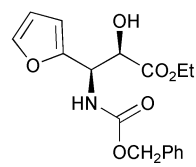
(7)

4.1.1 Amination of Alkanoic Acid Derivatives by Amines and Amine-related Reagents. Amino acid syntheses using an amination route, including a one-pot synthesis by reaction of 2-hydroxy-2-methoxyacetic acid methyl ester with benzyloxyamine and an alkyl radical using stannyl radical-mediated reaction,[55] the asymmetric synthesis of fluoro amines and amino acids using reducing agent-free, reductive amination of fluorocarbonyl compounds in three simple steps,[56] and the synthesis of chiral C-protected α-amino aldehydes of high optical purity[57] have been reported. The synthesis of enantiometrically pure α-amino acid derivatives from aldimines and tributyltin cyanide or achiral aldehydes, amines and hydrogen cyanide using a chiral zirconium catalyst,[58] and a convenient synthesis of the new sugar amino acid, 3-aminomethyl-3-deoxy-1,2-*O*-isopropylidene-α-D-ribo-hexofuranuronic acid,[59] have been described. It has been demonstrated that furyl and thienyl acrylates (**8**) (X = O, S; R = H, Me) could be subjected to aminohydroxylation with high selectivity, but pyrrolyl acrylates resist aminohydroxylation under the present reaction conditions. The resulting amino hydroxylation products (**9**) were readily converted to β-hydroxy-α-amino acids.[60]

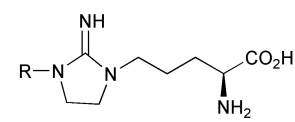
Guanidinium- and amine-containing amino acids based on a proline or alanine scaffold have been prepared. The guanidinium compounds were best prepared using 1*H*-pyrazole-1-carboxamidine hydrochloride as the guanidinating reagent, and the installed guanidino-group protected with Pmc. The resulting amino acids were incorporated into oligopeptides and tested for Tat-TAR interaction.[61] A method for the synthesis of ethylene-bridged (N^{δ} to N^{ω}) analogues of arginine (**10**) has been given. The initial step of the synthesis involved



(8)



(9)



(10)

the preparation of (*S*)-2-azido-5-bromopentanoic acid.[62] Large-scale syntheses of unnatural amino acids have been achieved by amination of keto acids using transaminases in a whole cell biotransformation,[63] and a convenient, scaleable process for the preparation of substituted phenylglycines by a modified Strecker reaction. Bisulfite-mediated addition of benzylamine and cyanide anion to substituted benzaldehydes gave aminonitriles, which were hydrolysed to the N-protected amino acid. Debenzylation resulted in good yields of substituted phenylglycines.[64]

4.1.2 Carboxylation of Alkylamines and Imines and Related Methods. A catalytic enantioselective aza-Diels–Alder reactions of imines to give optically active non-proteinogenic α-amino acids has been described.[65]

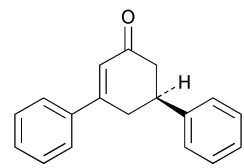
4.1.3 Use of Chiral Synthons in Amino Acid Synthesis. A stereocontrolled synthesis has been reported of enantiopure hydroxylamines having additional functionalities by reaction of chiral nitrones with a variety of nucleophiles. The hydroxylamines can be converted into amino acids and other nitrogenated compounds.[66] Cinchonidine and cinchonine, *N*-alkylated with Merrifield resin, have been employed as phase transfer catalysts for the enantioselective alkylation of enolates from *N*-(diphenylmethylene) glycine esters. Cinchonidine gave rise to (*S*)-isomers, whereas cinchonine gave (*R*)-isomers of amino acids.[67] The importance of a spacer in new chiral phase transfer supported catalysts used for the asymmetric synthesis of α-amino acids was studied. Polymer-supported cinchona alkaloid salts with different spacers were used as phase transfer catalysts in the asymmetric *C*-alkylation of *N*-diphenyl methylene glycine t-butyl ester for the synthesis of phenylalanine. Best results were obtained with cinchoninium iodide bound to polystyrene with a four-carbon spacer.[68] Syntheses of acyclic and heterocyclic (*S*)- and (*R*)-α-amino acids have been prepared from 1,5-dimethyl-4-phenylimidazolidin-2-one derived iminic glycinimides.[69]

Threo-β-hydroxy-1-glutamic acid derivatives with different carboxyl protecting groups have been synthesised using an aziridine-2-carboxylate as a chiral synthon in an aldol reaction[70] and protected (*S*)-4-carboxytetrahydro-1,3-oxazin-6-ones, synthesised by Baeyer–Villiger reaction on 4-ketoproline, have been developed as chiral templates in the synthesis of β-substituted aspartic acids.[71] α,α-Disubstituted α-amino acids have been synthesised asymmetrically under mild conditions using oxazinone and pyrazinone derivatives as chiral reagents,[72] while α,α-dialkyl-α-amino acids have been synthesised by enantioselective solid–liquid phase transfer catalytic alkylation of the aldimine Schiff base of amino acid *tert*-butyl esters with chiral quaternary ammonium bromides.[73]

Adducts from the diastereoselective Mannich-type reactions of aldehydes, 2-furylboronic acid and the chiral amine template (*S*)-5-phenylmorpholin-2-one have been used in the synthesis of enantiomerically pure D-α-amino acids.[74] L-2′-Bromo-phenylalanine and -tyrosine have been prepared using (−)-2,10-camphor sultam as a chiral auxiliary.[75] A copper(II) (salen) complex was used as an asymmetric phase transfer catalyst for the *C*-alkylation of *N*-benzylidene alanine methyl ester in the synthesis of α-methyl-α-amino acids; the enantiomeric excess was up to 86%.[76] Asymmetric syntheses of (2*R*,3*R*)- and (2*R*,3*S*)-3-hydroxypipecolic acids have been reported; the key step was the addition of Buchi's Grignard reagent to a chiral serinal.[77]

γ-Fluorinated-α-amino acids have been synthesised using 2-hydroxy-3-pinanone as a chiral auxilliary.[78] The asymmetric synthesis of quaternary α-amino acids using D-ribonolactone acetonide as a chiral auxiliary is presented.[79]

The preparation of planar chiral mimetics and their use in the stereoselective catalysis of the addition of Et_2Zn to PhCHO resulting in (*R*)-PhCH(Et)OH is described.[80] A new route for the preparation of enantiomerically pure quaternary α-substituted serine esters, involving the diastereoselective functionalisation of an oxazolidine ester enolate having an exocyclic chiral appendage is given.[81] The asymmetric synthesis of an (*S*)-ornithine and a chiral 2-cyclohexenone (**11**) *via* an enantioselective Michael reaction using chiral ammonium salts is reported.[82]



(11)

An easy three step process for the synthesis of optically pure α-amino acid derivatives bearing a bulky α-substituent involving an external chiral ligand-mediated asymmetrical addition of phenyllithium to an anisidine amine, oxidative removal of a N-PMP group, and finally oxidative conversion of the Ph group to a carboxyl group is reported.[83]

4.1.4 Use of Rearrangements Generating a Carbon–Nitrogen Bond. Pentacoordinated phosphorus compounds of amino acids and nucleosides,[84] the synthesis of compounds with side chain C–P links[85] and the asymmetric synthesis of phosphorus analogues of amino acids[86] have been reviewed.

Trisubstituted-benzoyl aziridine carboxylates have been synthesised from α-alkyl-β-amino acids. Ring expansion or ring opening of these compounds lead to α-substituted α-hydroxy-β- and β-hydroxy-α-amino acids.[87] Syn and anti-β-substituted α-amino acids have been prepared by a nitrone cycloaddition approach.[88] Various fluorenyl imines undergo a catalytic asymmetric Strecker-type reaction with trimethyl silyl cyanide in the presence of a Lewis acid–Lewis base bifunctional catalyst and a catalytic amount of phenol. The products were converted to amino acids without loss of enantiomeric purity.[89] Oxidative

rearrangement of azabicyclo[2.2.1]heptenes with mCPBA generated the oxazabicyclo[3.2.1]octenes, precursors for hydroxylated cyclopentyglycines.[90]

O-Phosphoryl amino acid esters have been prepared from *N*,*N*-dialkylphosphoramidates and the side chain hydroxy groups of tyrosine, serine and threonine.[91] *N*-(*O*,*S*-dimethylthiophosphoryl)-α-amino acid esters have been prepared in high yield and optical purity, and tested for insecticidal activity.[92]

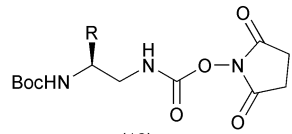
4.1.5 Other Rearrangements. Other rearrangement reactions have also been reported. The allylation of α-amino acid esters has been shown to give rise to intermediate quaternary ammonium salts which undergo proton abstraction to give ylides and [2,3]-Stevens sigmatropic rearrangement to give α-allyl α-amino acids,[93] allylic esters of TFA-protected amino acids underwent asymmetric Claisen rearrangements in the presence of cinchona alkaloids giving rise to γ,δ unsaturated amino acids in a highly stereoselective fashion.[94] The stereoselective synthesis of allylic amines by rearrangement, by the heating in xylene, under reflux, of allylic trifluoroacetimides allowed the synthesis of polyoxamic acid and derivatives of other α-amino acids.[95]

A process for the chelate–enolate Claisen rearrangements has also been reported for the asymmetric synthesis of unsaturated amino acids and peptides[96] and Claisen rearrangement methodology has also been reported for the synthesis of (2*S*,3*S*)-, (2*S*,3*R*)-substituted-L-glutamic acids starting from D-serine.[97] Unnatural α-amino acids were prepared when azetidin-2,3-diones have been reacted with primary amines in a one-step synthesis[98] and a novel synthetic route for the preparation of labelled amino acids by the rearrangement of α-aminocyclopropanone hydrate[99] and the synthesis of α-amino esters *via* the radical reaction of phenylsulfonyl oxime ethers on a solid support[100] have been described.

The synthesis of α- and β-amino acids by the isomerisation of aziridinyl ethers using superbases has been described.[101] The first racemic synthesis of the non-proteinogenic amino acid, (2*S*,3*R*,4*R*)-4-hydroxy-3-methyl-proline has been achieved *via* indolactonisation of an unnatural amino acid derivative. The relative stereochemistry was derived from an efficient silicon assisted aza-[2,3]-Wittig sigmatropic rearrangement.[102] Ring-opening of N-(PhF)serine-derived cyclic sulfamidate has been achieved with different nucleophiles (β-keto esters, β-keto ketones, dimethyl malonate, nitroethane, sodium azide, imidazole and potassium thiocyanate) to prepare a variety of amino acid analogues – two different pathways for ring opening were elucidated by the authors.[103] The application of Curtius rearrangements for the simple conversion of a number of N-Boc-protected β-amino acids into the corresponding *O*-succinimidyl-2-(tert-butoxycarbonylamino)ethylcarbamate derivatives (**12**, R = H, Me, I-Pr, CH_2Ph, CH_2CO_2Ph) has been reported.[104]

4.1.6 Amidocarbonylation and Related Multicomponent Processes. An overview of transition metal-catalysed amidocarbonylation together with views on future synthetic developments has been recently published.[105]

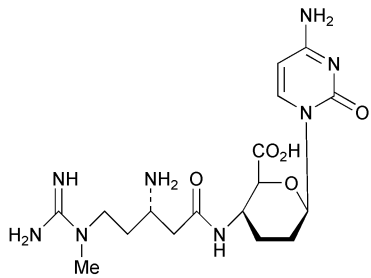
Biologically significant molecules have been prepared using this route of synthesis. N-Boc-iturinic acid and 2-methyl-3-aminopropanoic acid, compo-



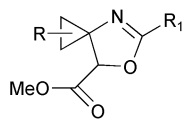
(12)

nents of the antifungal peptide iturin and depsipeptide cryptophycin, have been prepared by alkylation of functionalised succinic acid.[106] Blastidic acid, a component amino acid of the antibiotic blasticidin S (**13**), has been synthesised for the first time from α,γ-diaminobutyric acid by carbon-chain elongation, *N*-methylation and followed by amidination using *O*-methyl-*N*-nitroisourea.[107]

Galactopyranosyl azide was esterified with allyl tetramethylazelaoyl chloride and attached to Wang or Merrifield polymer through the other acid group. The azide was then reduced to amine using $HS(CH_2)_3SH$ and subjected to four-component reaction with aldehyde, isocyanate and HCO_2H. Acid hydrolysis yielded substituted phenylglycinamides.[108]



(13)



(14)

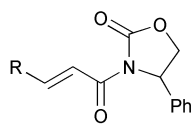
$\beta^{2,2}$-HBip, a biphenyl-substituted 3-amino-2,2-dimethylpropanoic acid has been prepared and converted into β-homo-peptides,[109] and 2-chloro-2-cyclopropylideneacetates, treated with carboxamides under basic conditions, was shown to undergo a domino transformation involving a Michael addition followed by an intramolecular nucleophilic substitution to afford 4-spirocyclopropane-annelated oxazoline-5-carboxylates [**14**, R = H, Et, $(CH_2)_2OCH_2Ph$, R_1 = Ph, nicotinic acid amide, furan-2-carboxamide, Me, Et, Pr, $C(CH_3)_3$]. These compounds are protected α-hydroxy-β-amino acids.[110] *Syn*-γ-hydroxy-β-amino acids have been prepared stereoselectively by iodolactonisation of 3-amino-4-pentenoic acid,[111] and successive protection, Arndt–Eisert reaction, Wolff rearrangement and deprotection of L-valine has been shown to lead to L-amino-4-methyl valeric acid hydrochloride.[112] The preparation of achiral and of enantiopure geminally disubstituted β-amino acids for β-peptide synthesis has been described.[113]

Previous reports in the literature that the treatment of *N*,*N*-dibenzyl amino alcohols with sulfonyl chloride lead to tetrahydroisoquinolines have been disproved. The products, which are intermediates in the synthesis of β-amino acids, are in fact β-chloro amines.[114]

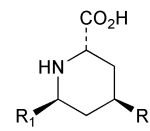
Aliphatic α-amino acids have been synthesised by one-pot reaction of aldehydes, KOH, ammonia and $CHCl_3$ in the presence of urea as a reversible phase transfer catalyst.[115]

4.1.7 From Glycine Derivatives and Imines of Glycoxylic Acid Derivatives. The synthesis of amino acids from glycine derivatives has been reported. The oxazolidinones (**15**) (R = various alkyl or aryl) served as Michael acceptors in addition reactions with achiral Ni(II) complexes of glycine Schiff bases. Deprotection of the appropriate resulting nickel complex (II) resulted in *e.g.* pyroglutamic acid in 96% yield with virtually complete stereoselectivity.[116]

Enantiopure 3-substituted pyroglutamic acids have been synthesised by Michael addition between 4*R*- or 4*S*-(*N*-*trans*-enoyl)oxazolidinones and the Ni(II)-complex of the chiral Schiff base of glycine with (*S*)-*o*-[*N*-(*N*-benzylprolyl)amino]benzophenone,[117] a Schiff base protected glycine, supported on poly(ethylene glycol), was reacted with electrophiles under microwave activation to produce α-amino acids,[118] and enantiomerically pure 2′,6′-dimethyltyrosine was synthesised by reaction of 4′-benzyloxy-2′,6′-dimethylbenzyl bromide with Ni(II) complexes of the chiral Schiff base of glycine with (*S*)-α-[*N*-(-benzylprolyl)amino]benzophenone.[119]



(15)



(16)

The diasteromerically pure pipecolic acids (**16**, R = H, Me, OH; R_1 = Pr, Et) have been synthesised from (*S*)-2-phenylglycinol[120] and imidazolidinone-bound glycine enolate derivatives were shown to undergo aldol condensation with aldehydes β-hydroxy-α-amino acids in a two-step process.[121]

N-Acyl-α-triphenylphosphonioglycinates, when reacted with carbon nucleophiles, gave rise to α-functionalised glycine derivatives.[122] New (*Z*)-α,β-didehydroamino acid derivatives with 3,5-dihydro-2*H*-1,4-oxazin-2-one structure have been synthesised by condensation of the chiral glycine equivalent with aldehydes in the presence of K_2CO_3 under mild solid–liquid phase transfer catalysis reaction conditions.[123]

Imines of glycoxylic acid derivatives have also been employed in the synthesis of amino acids. Alkylations and condensation reactions of both glycine and alanine imine have been studied and were shown to give rise to enolates which could be alkylated in a highly diastereoselective manner. The reactions gave rise to mono and dialkylated α-amino acids and heterocyclic derivatives.[124]

Amino acids derivatives have been synthesised *via* a scandium triflate three-component reaction of phenols, glyoxylates and amines,[125] pre-protected α,α-disubstituted amino acids have been prepared asymmetrically from tert-butylsulfinyl ketimines,[126] α- and β-amino acids by the stereoselective alkylation of chiral glycine and β-alanine derivatives[127] and after hydrolysis, α-methyl α-amino acids by the diastereoselective alkylation of an iminic alanine template with a 1,2,3,6-tetrahydro-2-pyrazinone structure.[128] The first asymmetric synthesis of α-amino acids based on diastereoselective carbon radical addition to glyoxylic imine derivatives is reported.[129]

4.1.8 From Dehydro-amino Acids Derivatives. The synthesis of β-substituted-α,β-dehydroamino acids by a Michael addition of heterocyclic nucleophiles to the methyl esters of *N-tert*-butoxycarbonyl-*N*-(4-toluenesulfonyl)-α,β-dehydroamino acids followed by a base-induced elimination of the 4-toluenesulfonyl group with the regeneration of the α,β-double bond has been reported,[130] and that of cyclic amino acid derivatives by a ring closing metathesis reaction on soluble poly(ethylene glycol)-supported allylglycine derivatives.[131]

Copper-promoted reaction of serine-derived organozinc reagents with allylic electrophiles gave Fmoc-protected amino acids ready for peptide synthesis[132] and new rhodium catalysts with unsymmetrical P-chirogenic bis(phosphino)ethanes, BisP*-Rh, were shown to exhibit very high enantioselectivity in the hydrogenation of α-dehydroamino acid derivatives.[133]

4.2 The Synthesis of Protein Amino Acids and Other Well-known Naturally Occurring Amino Acids. – Because of the commercial and biological importance of the protein amino acids and other naturally occurring amino acids, much work has been targeted towards the syntheses of these molecules. Work in the field has been the subject of a number of reviews, including the synthesis (and applications) of phenylalanine,[134] the synthesis of L-carnitine,[135] the syntheses (and biological evaluation of) (+)-lactacystin and its analogues,[136] and the asymmetric syntheses of α-substituted serines.[137]

Reviews have also been produced regarding the industrial production of some of these materials. The industrial production of D-alanine and D-tartaric acid using microorganisms has been reviewed,[138] as has the current status of lysine[139] and L-cysteine[140] production in China.

Large-scale processes have been developed for D-pyroglutamic acid production from L-glutamic acid by successive racemisation, resolution and dehydration.[141] and for the synthesis of L-DOPA tert-butyl ester, using catalytic enantioselective phase-transfer alkylation.[142]

The preparation (and applications) of phenylalanine[143] and L-threonine[144] have been reported. The synthesis has also been reported of tritium-labelled thyroxine and related compounds.[145] A study which may contribute to the explanation of the origin of life on earth has been published.[146] In this study, the thermochemical aspects of the conversion of the gaseous system CO_2–N_2–H_2O into a solid amino acid condensate in an electric discharge plasma are considered.

A number of enzymic syntheses have also featured in the literature including the enzymic synthesis of ^{32}P-labelled phosphoarginine,[147] the preparation and resolution of both epimers of L-cyclopentenylglycine (this method was used for preparation of ^{13}C-labelled compounds for use as tracers)[148] and the synthesis of L-tryptophan from L-cysteine and indole using the genetic engineering strain WW-ll.[149] The two isotopomers of L-phenylalanine, ^{13}C or ^{14}C labelled in the carboxyl group have also been synthesised enzymatically,[150] as has L-[4-^{13}C] aspartic acid.[151] [^{15}N]-D-isovaline was prepared from DL-[α-^{15}N]-α-aminoisovaleramide by enzymic resolution with *Mycobacterium neoaurum.*[152]

The synthesis of common naturally occurring amino acids and their derivatives has been widely reported. The effects of the reaction conditions and the

preparation methods of the catalyst and the related technology for the alkali water catalytic oxidation of ethanolamine to glycine using a Cu/ZnO catalyst have been studied.[153] Phenylglycine was synthesised in one pot from benzaldehyde, KOH, NH_4OH and $CHCl_3$ under the catalysis of a phase transfer catalyst and β-cyclodextrin,[154] and (*S*)-cyclohexyl glycine has been prepared in high yield by hydrogenation of (*S*)-phenylglycine using rhodium on carbon as the catalyst.[155] A new synthetic route for the preparation of *p*-hydroxyphenylglycine and some analogues from *p*-benzoquinone has been shown to achieve a diastereoselectivity of 60% using 8-phenylmenthyl acetate as the chiral auxiliary.[156]

The synthesis of enantiomerically pure (*S*)-phenylalanine[157] and the synthesis of phenylalanine from benzylidene glycinate *via* C-alkylation using microwave irradiation and phase-transfer catalysis have been described.[158] An efficient method has been reported for the conversion of β-phenylisoserine to β-hydroxyphenylalanine derivatives *via* aziridines,[159] and highly functionalised phenylalanine derivatives have been prepared using cross-enyne metathesis and Diels–Alder addition as key steps.[160] L-(+)-Homophenylalanine hydrochloride has been synthesised in 55% yield with 99% enantiomeric excess from *N*-phthaloyl-L-aspartic acid.[161]

The syntheses of [ring-^{14}C]-L-tyrosine from [U-^{14}C]-phenol,[162] and other isotopically-labelled amino acids, including 2,3,4,2′,3′,5′,6′-$^{2}H_7$-L-tyrosine[163] and 3,3,4,4,3′,3′,4′,4′-$^{2}H_8$-homocystine,[164] have been reported.

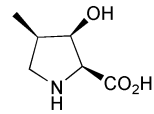
O-Phosphoryl amino acid esters have been prepared from *N*,*N*-dialkylphosphoramidates and the side chain hydroxy groups of tyrosine, serine and threonine.[165] D,L-Serine has been prepared in 89% yield with 92.0% purity in two steps from α-chloro-β-aminoproprionitrile hydrochloride.[166]

A Co(III) imino acid complex has been used for the stereospecific incorporation of deuterium into the α- and β-carbon atoms in α-amino acids[167]and *N*-(α-stannylalkyl)oxazolidinones, prepared in three steps from aldehydes, have been shown to undergo tin-lithium exchange to give *N*-(α-lithioalkyl)oxazolidinones. The latter undergo carboxylation to diastereopure *N*-(α-carboxyalkyl)oxazolidinones. Birch reduction of the oxazolidinone moiety then yielded amino acids; and so this rapid method is useful for the preparation of ^{11}C-amino acids.[168] The synthesis of isotopically labelled L-α-amino acids with an asymmetric centre at C-3 has been reported and the method can be adapted to allow the introduction of a label at each site of L-valine.[169] A synthesis of γ-oxo-α-amino acids from polymer-supported α-imino acetates has been reported.[170]

α-Hydroxy and α-amino acids have been prepared by the nucleophilic ring opening of gem-dicyanoepoxides by LiBr or Li_2NiBr_4 in the presence of hydroxylamines *via* α-halohydroxamic acids.[171]

A novel synthetic protocol for enantiopure substituted prolines[172] and the diastereoselective synthesis of (2*S*,3*S*,4*S*)-3-hydroxy-4-methylproline (**17**), a common constituent of antifungal cyclopeptides, from unsaturated lactams are described.[173]

Stereoselective syntheses of (*S*)-5-hydroxynorvaline from glutamic acid,[174] (−)-*N*-Boc-AHPPA[175] and both enantiomers of *trans*-4-pipecolic acid and the



(17)

natural product (−)-SS20846A have been reported.[176] In the latter study the stereochemistry of key intermediates was established by X-ray diffraction analysis.

A large number of amides and esters of glutamic acid have been prepared using chemoselective ring opening of N-Boc pyroglutamic-Wang resin by heteronucleophiles.[177] and a simple transformation of L- and D-glutamic acids into all four possible stereoisomers of 5-hydroxylysine has also been reported.[178]

(−)-Kainic acid has been synthesised using a titanium-mediated cyclisation sequence starting from L-serine,[179] by a sulfanyl radical addition–cyclisation–elimination reaction of diallylamines in the presence of thiophenol and AIBN[180] and by employing a concurrent Chugaev syn-elimination and intramolecular ene reaction from (+)-*cis*-4-carbobenzoxyamino-2-cyclopentenol.[181] A range of 4-arylsulfanyl-substituted kainoid amino acids have been synthesised from *trans*-4-hydroxy-1-proline.[182]

Synthetic routes have also been reported for a phosphonic analogue of (−)-allo-norcoronamic acid.[183] (+)-alloisoleucine,[184] optically pure L-homocysteine from L-methioine (in an easy two step synthesis)[185] and the naturally occurring (2*S*,3*R*, 4*S*)-3,4-methanoproline and its synthetic constitutional isomers.[186] An efficient method has also been developed *via* the Schollkopf chiral auxiliary for the asymmetric syntheses of iso-, homo- and benzo-tryptophan.[187]

Stereoselective conjugate addition of lithiated (*S*)-(α-methylbenzyl)benzylamide to (*E*)-7-(tosyloxy)hept-2-enoic acid tert-butyl ester, followed by deprotection, gave protected β-homolysine of greater than 99% enantiomeric purity.[188] The syntheses of the following materials have also been reported: (±)-homohistidine was prepared from the readily available urocanate;[189] L-glutamine from L-glutamic acid (*via* a three step synthesis);[190] L-cysteic acid (by electrooxidation);[191] *cis*-3-hydroxy-L-proline from β-alanine;[192] and (3*S*,4*R*)-3,4-dimethylglutamine, by asymmetric Michael addition and electrophilic oxidation – three adjacent stereogenic centres were generated simultaneously in this synthesis using a camphorsultam chiral auxiliary.[193]

Resin bound *N*-acylated amino acid aldehydes were converted in a single step to a-hydroxy phosphonates by a Pudovik reaction and in six steps to hydroxystatine amides, useful for constructing multiple aspartic acid transition state isosteres.[194] The synthesis of a series of L-alanine hydroxamate sulfonylated derivatives as protease inhibitors has been reported.[195] The compounds were tested as inhibitors of *Clostridium histolyticum* collagenase.[196] Protected 4-hydroxypyroglutamic acids were prepared by 1,3-cycloaddition of furfuryl nitrones with acrylates.[197] The stereoselective synthesis of both enantiomers of *threo*- and *erythro*-β-hydroxy norvaline, involving the addition of different organometallics to (*S*)-serine derivatives, has been reported,[198] whereas three approaches to the synthesis of L-leucine selectively labelled with carbon-13 or deuterium in either

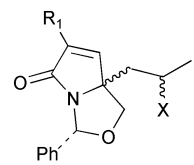
diastereotopic methyl group have been followed. In all three methods the stereogenic centre at C-2 was created with total stereocontrol.[199] A new method has been reported for the synthesis of 2-phenylproline by intramolecular cyclisation of *N*-(3-chloropropyl)-α-phenylglycine under phase transfer catalysis conditions[200] and studies are reported on the progress in the no-carrier-added radiosynthesis of [^{18}F]-fluoroarginine for use as a probe for nitric oxide synthetase activity.[201]

L-cysteic acid has been synthesised by indirect electrooxidation and its applications have been discussed,[202] and the synthesis and structures of Fe(Cysteine)$_{1.5}$H$_2$O and Na$_2$[Fe(Cys)$_2$]H$_2$O have been reported.[203]

4.3 Synthesis of α-Alkyl-α-Amino Acids. – The synthesis of α-methyl-L-tryptophan**,** from an indolylmethylimidazolidinone using LDA,[204] α,β-dialkyl-α-phenylalanines, *via* direct alkylation of a Ni(ll)-complex of a Schiff base of alanine with (*S*)-*o*-[*N*-(*N*-benzylprolyl)-amino]benzophenone with racemic α-alkylbenzyl bromides[205] and (*S*)-cyclohexylglycine**,** by the hydrogenation of (*S*)-phenylglycine using rhodium on carbon as a catalyst,[206] have been described.

Diethyl α-acetamido α-alkylated malonates[207] and new amino acid, tosyl and phthalyl amino acid derivatives of 3-carbethoxy methyl-7-hydroxy-4-methylcoumarin[208] have been synthesised. In both cases, the structures of the final products were confirmed.

In the presence of a Lewis acid, a Michael-type reaction of (**18**) with nitro olefins gave good yields of pyrrolo-oxazolones. These compounds were transformed into α-branched serine derivatives.[209]



(18)

4.4 Synthesis of α-Amino Acids Carrying Alkyl Side Chains and Cyclic Analogues. – Considerable interest has been shown in the synthesis of α-amino acids with alkyl side chain and cyclic analogues and a review of the chemistry of one such, 2-aminocyclopentanecarboxylic acid, has been published.[210]

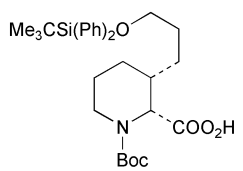
α-Methyl-α-amino acids have been prepared by the Ugi reaction using *Z*-L-Lys(*Z*)-OH, benzylamine, alkyl methyl ketone and cyclohexyl isocyanide, following hydrolysis of the resulting diastereomeric dipeptides,[211] and α-alkyl-α-amino acids were obtained by the hydrolysis of the α-alkyl-α-amino nitriles resulting from the addition of Et$_2$AlCN and isopropyl alcohol to *N*-sulfinyl imines in an asymmetric Strecker synthesis.[212] α,α-Disubstituted amino acids have been synthesised, also using an asymmetric Strecker synthesis, with alkyl halides or aldehydes,[213] and an efficient enantioselective synthesis of α-methylaspartic acid and 3-amino-3-methylpyrrolidin-2-one has been described.[214]

Several groups of workers have conducted studies on pipecolic acid, including the synthesis of C-6 substituted pipecolic acid derivatives using an intramolecu-

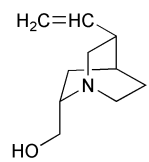
lar Mannich-type reaction,[215] the asymmetric synthesis of all four isomers of 4-hydroxypipecolic acid from δ-amino-β-keto esters,[216] the synthesis of a novel constrained pipecolic acid (**19**) in seven steps in 86% yield with 94% optical purity from $TBDPSO(CH_2)_3$,[217] and the preparation of 2,3-methanopipecolic acid from L-lysine *via* 2,3-didehydroo-1,2-bis(methoxycarbonyl)-6-methoxypiperidine. The 6-methoxy group acted as a chiral auxiliary.[218]

The preparation of a series of carbocyclic α-amino acids from four different racemic 2-alkylated cyclopentanones and (*R*)-1-phenylethylamine as the chiral auxilliary by means of an asymmetric Strecker synthesis, the stereoselectivity being influenced by the solvent and by the size of the cyclopentanone C-2 substituent,[219] and the synthesis of novel bridged bicyclic α-amino acid esters (**20**) and key derivatives from quincorine and quincoridine,[220] have been reported.

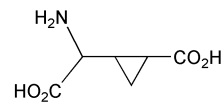
Stereoselective intramolecular conjugate addition of the benzamide group to cylclohexenone, promoted by Lewis acid and subsequent transformations, has been used to synthesise conformationally constrained hydroxyphenylcyclohexane α-amino acids[221] and 1-aminocyclopropane carboxylic acids and bicyclic α-amino acids have been prepared from a chiral glycine equivalent with a 1,2,3,6-tetrahydropyrazine-2-one structure.[222] The syntheses of α-amino acids with a cyclohexene substituent have been reported[223] and a methodology has been presented for the synthesis and conformational analysis of azacycloalkane amino acids as conformationally constrained probes for mimicry of peptide secondary structures.[224] A protected form of (*R*,*R*,*R*)-2,5-diaminocyclohexanecarboxylic acid has been synthesised and found to function as a building block for helix-forming β-peptides.[225]



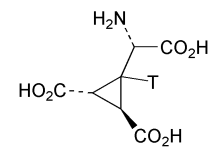
(19)



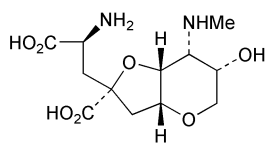
(20)



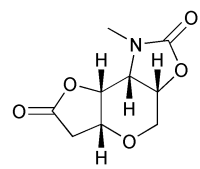
(21)



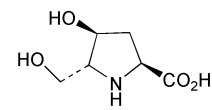
(22)



(23)



(24)



(25)

An improved synthesis of *N*-Boc-*O*-cyclohexyl tyrosine has been reported.[226] The stereoselective syntheses of two carboxycyclopropylglycines (**21**) based on the stereochemical control of the 1,3-dipolar cycloaddition of diazomethane provided by the 4-methyl-2,6,7-trioxabicyclo[2.2.2.]-orthoester function on chiral *E*- or *Z*-3,4-L-didehydroglutamates have been detailed,[227] and the syntheses of (2*S*,1′*R*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine[228] and (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine[229] have been reported. A syn-

thesis of (2*S*,2′*R*,3′*R*)-2-(1′-[^{3}H] ,2′,3′-dicarboxylcyclopropyl)-glycine ([^{3}H]-DCG-IV) (**22**) has also been reported.[230]

Other relevant syntheses reported are those of (−)-dysiherbaine (**23**), a novel neuroexitotoxic amino acid,[231] which has also been synthesised *via* the key intermediate (**24**) which was prepared in seven steps from (2*E*,5*E*)-($PhCH_2CH{:}CH)_2CHOH$,[232] (2*S*,4*R*)-4-hydroxypipecolic acid,[233] the *Z*-isomers of cyclobutane dehydroamino acids from (−)-α-pinene and (−)-verbene,[234] (2*S*,4*S*,5*R*)-(−)-bulgecinine (**25**)[235] and the precursors to vicinal *cis*-dihydroxy-1-aminocyclopentane- and -cyclohexanecarboxylic acid methyl esters which give rise to enantiomerically pure products.[236]

A mixture of the four stereoisomers of *N*-carbamoyl-β-methylphenylalanine was hydrolysed and separated enzymatically to give the four isomers of β-methylphenylalanine in high optical yield[237] and a process is reported for the synthesis of both stereoisomers of 1-amino-4-hydroxycyclohexane-1-carboxylic acid through selective transformations of the functional groups of the corresponding enone cycloadduct provided by the Diels–Alder cycloadditions of Danishefsky's diene to methyl 2-acetamidoacrylate.[238]

Synthetic routes have also been reported for 4-alkyl and 4-cinnamyl glutamic acids, which were subsequently shown to be potent GluR5 kainate receptor agonists,[239] *N*-Fmoc 4-(2′-(di-tert-butyl-malonyl)-phenylalanine – a key step being the introduction of chirality using the Williams auxiliary (benzyl (2*R*,3*S*)-(−)-6-oxo-2,3-diphenyl-4-morpholinecarboxylate),[240] 2-amino-3-hydroxynorbornanecarboxylic acid derivatives containing a conformationally constrained serine skeleton, by cycloaddition of cyclopentadiene with an oxazolylidene derivative,[241] all four stereoisomers of 2,3-methanoleucine,[242] *N*-(2*H*-azirinyl)-L-prolinates which are heterospirocyclic dipeptide synthons,[243] and 2,3-methanoamino acids**,** prepared from ethyl 3,3-diethoxypropionate by titanium(IV)-mediated cyclopropanation using Grignard reagents.[244]

α-Substituted pyroglutamates have been prepared from tributyltin hydride mediated cyclisation of dehydroalanine,[245] tetralin-based constrained α-amino acid derivatives *via* [4 + 2]-cycloaddition reaction as a key step[246] and α-CF_3-substituted α-amino phosphonates with two alkene chains, 1,7-dienes and 1,8-dienes have been synthesised by nucleophilic addition to PG-$N{=}C(CF_3)P(O)(OR_2)$. Treatment with a ring closing metathesis catalyst yielded P-containing analogues of dehydropipecolinic and tetrahydroazepin-2-carboxylic acids.[247]

The synthesis of 3,5-di-tert-butyltyrosine from tyrosine ethyl ester by the action of isobutylene in methylene chloride in the presence of sulfuric acid has been described[248] and a general method has been devised for the one-step preparation of 4-(acylamino)piperidine-4-carboxylate esters from the corresponding α-amino acids.[249]

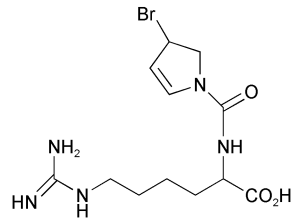
Two procedures for the enantioselective synthesis of protected forms of (3*R*,5*R*)-5-hydroxypiperazic acid have been reported.[250]

The synthesis of α-amino alkanephosphonic acids,[251] and a modified Arndt–Eistert procedure for synthesis of homo-chiral *N*-alkoxycarbonyl α-ethyl aminoadipates and ethyl 6-oxopipecolates have been described.[252] New chiral

amino acids have been synthesised from *cis*-caran-*trans*-4-one and (−)-menthone *via* appropriate lactams.[253]

4.5 Models for Prebiotic Synthesis of Amino Acids. – Theoretical study of the addition of hydrogen cyanide to methanimine in the gas phase and in aqueous solution has been conducted,[254] and the abiotic synthesis of amino acids in simulated primitive environments by radiation has been studied.[255] The stereoselective approach and mechanistic aspects relating to access to proline chimeras have been considered as part of a series of studies looking at pyrrolidines bearing a quaternary α-stereogenic center.[256]

N^{α}-(4-bromopyrrolyl-2-carbonyl)-L-homoarginine (**26**), a natural product from the sponge *Agelas wiedenmayeri*, has been synthesised from lysine. The compound is suggested as a key intermediate in the biosynthesis of pyrrole-imidazole alkaloids.[257]



(26)

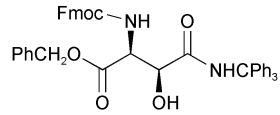
4.6 Synthesis of α-(ω-Halogeno-alkyl) α-Amino Acids. – A new review of the asymmetric synthesis of fluoro amino acids has been published[258] and the review originally published in 1997[259] has been updated.[260] The syntheses of fluoro and difluoroalanines, using tris(diethylamino)-*N*-methylphosphazene for the fluoromethylation of diethyl *N*-acetylaminomalonate by CH_2BrF or $CHClF_2$ in DCM,[261] 3,3-difluoroserine and -cysteine derivatives *via* Mg(0)-promoted selective C–F bond cleavage of trifluoromethyl imines,[262] *cis*-4-[^{18}F]fluoro-L-proline and *trans*-4-[^{18}F]fluoro-L-proline have been synthesised *via* a semi-automated, NCA procedure using the General Electric FDG microlab, a system employing a quaternary 4-aminopyridinium resin to effect F-18 fluorination,[263] and α-difluoromethyl prolines and α-aminoadipic acids by trapping reactions of *in situ* generated *N*-protected α-methyl difluoroalaninyl radicals[264] have been reported.

A facile and stereoselective synthesis of non-racemic trifluoroalanine has been reported.[265] The first synthesis of a totally orthogonal protected α-(trifluoromethyl)- and α-(difluoromethyl)arginine has been reported.[266] The novel synthesis of 5-chloro- and 5-bromo-tryptamines and -tryptophans and its application to the synthesis of bromochelonin has been reported.[267]

4.7 Synthesis of α-(ω-Hydroxyalkyl) α-Amino Acids. – A highly stereoselective synthesis of γ,δ-unsaturated amino acids involving the asymmetrical Claisen rearrangement of allylic esters of TFA-protected amino acids in the presence of

cinchona alkaloids has been reported.[268]

The synthesis of (2*R*,3*S*)-β-hydroxy leucine and all four isomers of β-phenyl serine, using the sulfinimine-mediated Strecker synthesis,[269] 3,4-dihydroxyprolines by application of an L-threonine aldolase-catalysed aldol reaction[270] and (**27**), from methyl (*E*)-4-methoxy cinnamate *via* the Sharpless asymmetric aminohydroxylation reaction,[271] have been reported.



(27)

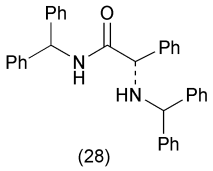
β-Hydroxyaspartic acid derivatives have been synthesised and tested as glutamate transport blockers.[272] On addition of Et_2AlCN/I-PrOH, masked oxo sulfinimines gave α-amino nitriles that afforded oxo α-amino acids on hydrolysis.[273]

4.8 Synthesis of *N*-Substituted α-Amino Acids. – A general route for the solid phase synthesis of *N*-substituted α-amino acids using Fukuyama's sulfonide protecting group has been reported.[274] More specific synthetic methods for *N*-methyl-α-amino acids from *N*-carbamoyl α-amino acids *via* oxazolidinones,[275] *N*-hydroxyamino acids *via* the selective *N*-hydroxylation of *N*-Boc protected primary amino acid esters with methyl(trifluoromethyl)-dioxirane under mild conditions[276] and *N*(σ)-alkyl histamine and histidine derivatives through efficient alkylation followed by deprotection using activated silica gel[277] have been presented. The synthesis of derivatives of arginine containing several chiral centers has been reported.[278]

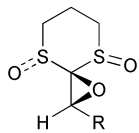
Reaction of α-amino acids with ketones under hydrogenation conditions using 20% $Pd(OH)_2/C$ gave *N*-monoalkylated amino acids; methylation under the same conditions gave *N*,*N*-dialkylated derivatives.[279] A series of *N*-formyl-*O*-acyl-β-phenylserine derivatives has been prepared by the interaction of *N*-acyl-β-phenyl serine ethyl esters with formic acid in the presence of HF[280] and a series of *O*-(4-amidinophenoxy)alkyl-*N*-substituted tyrosine methyl esters have been synthesised by etherification of 4-cyanophenol with dihaloalkanes in NaOH and conversion of the cyano group to amidine. Their activities were tested against Adp-induced platelet aggregation.[281] γ-Oxygenated *N*-phthalimido glutamic acid derivatives have been prepared by a mild version of the LemieuxJohnson olefin cleavage followed by peroxide mediated dialdehyde oxidation,[282] and the syntheses of (*S*)-proline derivatives which contain a 2,4,6-trimethyl-, 4-tert-butyl- or pentamethylbenzyl-substituent on the nitrogen atom have been reported.[283]

α-Amino amides such as (**28**) have been synthesised by epoxidation of alkylidenedithiane dioxides (R = Ph, 4-ClC_6H_4, 4-$O_2NC_6H_4$, c-C_6H_{11}) *via* the spirocyclic oxiranes (**29**).[284] A number of materials in this group show biological activity. The synthesis has been reported of the complexes of the Schiff bases *N*-vanillin-α-phenylalanine(L_4) with lanthanide(III) ions[285] and these materials have been shown to have antitumour activity. Synthetic routes have also been presented for ^{11}C-labelled *N*-methylaminoisobutyric acid, an achiral synthetic

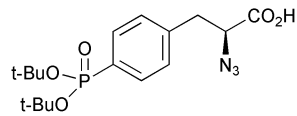
amino acid which has proved useful for *in vivo* studies of amino acid transport systems in man,[286] N^G-(1-iminoethyl)phosphalysine derivatives, which act as inhibitors of nitric oxide synthase,[287] and *N*-(hydroxyaminocarbonyl)phenylalanine, an inhibitor for carboxypeptidase A.[288]



(28)



(29)



(30)

Also reported are syntheses for N^G-(4-nitrobenzenesulfonyl)-L-arginine,[289] *N*-benzyl-(hydroxyphenyl)glycines *via* a Mannich reaction of phenols with glyoxylic acid and benzylamine,[290] *N*-formamidinylamino acids from amino and formamidinesulfonic acids,[291] *N*-acetyl-L-cysteine**,** using a new synthetic method using acetic anhydride,[292] and *N*-benzoxycarbony-*S*-phenyl-L-cysteine from L-cysteine, by substituting with benzenediazonium chloride and acylating with CbzCl.[293] 2-Nitrofluoren-9-ylmethyloxycarbonyl amino acids have been prepared by the reaction of 9-fluorenylmethyloxycarbonyl amino acids with 100% nitric acid in DCM,[294] as well as mesityl-substituted amino acids.[295] A large scale production of N^{ε}-trifluoroacetyl-L-lysine, a starting material for the production of lysinopril, is given.[296]

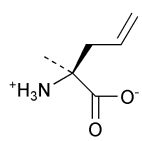
The first synthesis of one of the four possible stereoisomers of 3,4-dihydroxy-L-glutamic acid ((3*S*,4*S*)-DHGA) is reported[297] and four and seven step, respectively, processes are reported for the synthesis of N-Boc-protected (4*R*,3*R*)- and (2*R*,3*S*)-3-fluoroprolines from (2*R*,3*R*)- and (2*S*,3*S*)-3-fluoroprolines.[298]

N^{α}-Lauroylarginyl methyl ester hydrochloride, a cationic surfactant, has been prepared using highly concentrated water-in-oil emulsion as a new reaction media.[299]

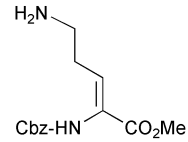
A series of novel *N*-[α-(isoflavone-7-*O*-)acetyl]amino acids methyl esters were prepared from chloroacetyl amino acids under mild conditions,[300] *N*-thiazolyl α-amino acids derivatives were readily synthesised from α-amino acids and α-bromo ketones,[301] and the preparation is reported of a new α-azido phosphotyrosyl mimetic (**30**) using a Heck reaction.[302]

The synthesis of the *N*-aryl amino acids has been reported. The coupling of cinnamic and 3-(2-furyl)acrylic acids with amino acids esters followed by saponification and amidation gave rise to *N*-(cinnamoyl)- and 3-(2-furyl)acryloyl amino acids,[303] and the synthesis and biological activity of *N*-aryl-β-alanines and the products of their cyclisation has been reported.[304]

α-Siloxyamides, specifically (−)-betsatin, have been synthesised from H-$C(CN)_2O$-$SiMe_2$.tert-Bu, a carbonyl compound and primary amine, mixed together in acetonitrile,[305] *N*-methylaspartic acid derivatives and their homologues were obtained by a stereoconservative one-pot procedure from hexafluoroacetone-protected aspartic and glutamic acid,[306] and the synthesis of *N*-phosphonamidothionate derivatives of glutamic acid has been detailed.[307]



(31)



(32)

4.9 Synthesis of α-Amino Acids Carrying Unsaturated Aliphatic Side Chains. – Syntheses have been reported for the α-*C*-methylated side chain unsaturated α-amino acid Mag (**31**), using a chemo-enzymic method,[308] predominantly *Z*-dehydroamino acids from ethyl *N*-Boc- and *N*-*Z*-α-tosylglycinates and nitro compounds,[309] and twenty four 4-alkylidene glutamic acids.[310] The latter were tested as GluR5 agonists. The synthesis of non-proteinogenic amino acids *via* ester enolate Claisen rearrangements is reported.[311]

A synthetic route for the conversion of a (*Z*)-α,β-didehydroornithine (**32**) derivative to α,β-didehydrokyotorphin,[312] the preparation and properties of model dehydroalanine derivatives,[313] and the preparation of α,β-dehydro amino acids, from the reaction of β-hydroxy-α-amino esters with dichloroacetyl chloride in the presence of base,[314] have been reported.

The synthesis of vinyl amino acids is discussed[315] and the installation of the (1-fluoro)vinyl trigger for β,ω-unsaturated amino acids is specifically discussed.[316] A synthetically malleable class of quaternary α-(2-trialkylstannyl)vinyl amino acids that could be used as building blocks in *de novo* peptide design have been described[317] and a generalised synthesis is reported of higher L-α-vinyl amino acids has been given. The side chain is introduced by alkylation of a chiral vinylglycine-derived dianionic dienolate bearing the D'Angelo auxiliary, which can be recovered,[318] and a synthetic route for the preparation of a variety of enantiomerically enriched β,γ-unsaturated α-amino acids by olefination of a Cbz-protected serine aldehyde equivalent has been presented.[319] L-3,4-Didehydrovaline, an important constituent of the antibiotic phomopsin A, has been synthesised from D-serine in 31% yield.[320] The synthesis of γ,δ-didehydrohomoglutamates by the phosphate-catalysed γ-addition reaction to acetylenic esters has been reported[321] and the stereoselective synthesis of *Z*-alkoxycarbonylamino-4-phenylbut-2-enoate is reported.[322] A molybdenum-catalysed regioselective synthesis of α-stannylated allylic esters, suitable substrates for chelate Claisen rearrangements[323] and a one pot reaction of *N*-benzylhomoallylamine with glyoxylic acid monohydrate in methanol to give *N*-benzylallylglycine have been reported.[324] *N*-(5-acetyl-6-methyl-2-oxo-2*H*-pyran-3-yl)-benzamine and *N*-(5-benzoyl-6-methyl-2-oxo-2*H*-pyran-3-yl)benzamide were reacted with various hydrazines to give the corresponding α,β-didehydro-α-amino acid derivatives.[325]

4.10 Synthesis of α-Amino Acids with Aromatic or Heteroaromatic Couplings in the Side Chain. – The synthesis of unnatural amino acids by reduction and ozonolysis of aromatic amino acids has been reviewed,[326] and two reviews on the synthesis of conformationally constrained aromatic amino acids have been published.[327, 328]

A significant body of work has been published regarding the synthesis of α-amino acids with side chains incorporating aromatic groupings in the side chain. The formation of optically active aromatic α-amino acids by catalytic enantioselective addition of imines to aromatic compounds[329] has been reported and an automated synthesis apparatus, developed for L-[3-^{11}C] aromatic amino acids, has been described.[330] The synthesis of indane-based unusual α-amino acid derivatives under phase-transfer catalysis conditions has been reported.[331]

The cross-coupling of aryl boronic acids and alkanethiols mediated by copper(II) acetate and pyridine in anhydrous DMF gave aryl alkyl sulfides; this method can be applied to the synthesis of aryl sulfides of cysteine,[332] while an organoborane, prepared from protected allylglycine, was used in a Suzuki cross-coupling reaction with olefinic aromatic and heteroaromatic bromides to give a range of novel α-amino acids.[333]

The syntheses of optically active phenylglycine derivatives, from *S*-(+)-*N*-(benzylidene)-*p*-toluenesulfinamide using Lewis acids and tert-amines,[334] (*S*)-*N*-tosyl-1-naphthylglycine using a Sharpless asymmetric aminohydroxylation as a key step,[335] and (*S*)-β^2-homoarylglycines[336] have been described. (*R*)- and (*S*)-α-Amino alcohols and α-amino acids, including 4-methoxyhomophenylalanine with a variety of unnatural side chains, were synthesised *via* palladium-catalysed cross-coupling Suzuki reactions.[337] Enantiomerically pure *trans*-cinnamylglycine and -alanine has been prepared by reaction of cinnamyl halides with Ni(II) complexes of chiral Schiff bases of glycine and alanine. The simplicity of the reactions and the high stereochemical outcome make the procedure suitable for large-scale preparations.[338]

The synthesis of 3-(3′-fluorenyl-9′-OXO)-L-alanine, a novel photoreactive conformationally constrained amino acid[339] and photoactivable 4-aroyl-1-phenyl alanines from 4-iodo-1-phenylalanines using a carbonylative Stille cross-coupling reaction[340] has been reported.

Other examples of α-amino acids of this group for which synthetic routes have been reported are all four isomers of α-methyl-β-phenylserine, synthesised from (*S*)- and (*R*)-*N*-Boc-*N*,*O*-isopropylidene-α-methylserinals.[341] The synthesis of α-amino acids with heteroaromatic groupings in the side chain has been reported.

The synthesis of novel heterocyclic substituted α-amino acids using α-amino acid alkynyl ketones as reaction substrates is reported.[342] A number of these have nitrogen-containing rings as part of the side chain. Reactions of 5-substituted (*S*)-1-acyl-3-[(*E*)-(dimethylamino)methylidene]pyrrolidin-2-ones and (*S*)-3-[(*E*)-(dimethylamino)methylidene]tetrahydrofuran-2-ones with amines have been reported. Preparation of intermediates in the ring switching synthesis of hetero-arylalanine- and hetero aryllactic acid derivatives and their analogues have also been detailed.[343] A short and effective enantioselective synthesis of β-heterocyclic amino acid derivatives is described using a kinetic resolution by an acylase from *Aspergillus* species.[344] Syntheses are presented for novel quinolyl glycines, prepared stereoselectively from 2-aminothiophenol and chiral acetylenic ketones which contained a masked α-amino acid functionality, the resulting benzo[*b*][1,4]thiazepine derivatives being converted to quinolyl glycines,[345] phenylalanine and phenylglycine derivatives, possessing a porphyrin moiety,[346]

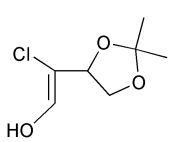
and acetyl-β-(1-azulenyl)-L-alanine in high yield by the malonic ester condensation procedure. This latter compound is a potential blue-coloured fluorescent tryptophan analogue.[347]

Starting from L-serine, pyrazolyloxazolidines have been prepared and transformed into chiral α-amino acids containing a pyrazole ring.[348] The syntheses of β-1*H*-1,2,3-triazol-1-yl and β-2*H*-1,2,3-triazol-2-yl α,β-unsaturated α-amino acid derivatives by an isomerism reaction,[349] a range of novel heterocyclic α-amino acids by the reaction of diamines and amidrazones with α-amino acid vicinal tricarbonyl reactive substrates,[350] and optically active amino acid derivatives of methylated 5-amino-azaheterocycles[351] have been reported.

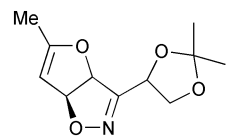
Cycloaddition of trimethyltin azide with the nitrile group of 4-cyanophenylalanine analogues gave 4-(tetrazol-5-yl)phenylalanine,[352] and pyri-midine and purine amino acids prepared by conjugate radical addition of *N*-(2-iodoethyl)- and *N*-(2-iodopropyl)-pyrimidines and purines with an optically active oxazolidinone[353] have been outlined.

Heterocycles containing both sulfur and nitrogen have also been utilised as parts of side chains. Thiazole- and oxazole-containing amino acids and peptides were prepared using amino acids as educts.[354] The synthesis and resolution of 3-(4-thiazolyl)-D,L-alanine has been reported.[355]

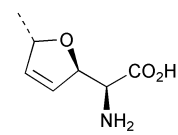
Thiazole containing non-proteinogenic amino acids were synthesised and tested for anti-bacterial activity[356] and orthogonally protected 3-(1-aminoalkyl)isoxazole-4-carboxylic acid has been prepared by 1,3-dipolar cycloaddition of an α-aminonitrile oxide with an enaminoester dipolarophile. The resulting unnatural amino acid, after deprotection, was used as peptide bond replacement.[357] Those analogues containing both oxygen and nitrogen have similarly been used. Analogues of glutamic acid with conformationally restricted structures, 3-carboxyisoxazolinylprolines and related compounds have been synthesised and tested for glutamate receptor activity.[358] A novel isoxazole derivative, *O*-(5-isoxazolyl)-L-serine was synthesised by a Mitsunobu reaction of isoxazolin-5-one with *N*-Boc-L-serine tert-butyl ester and subsequent deprotection of the coupling product is reported.[359]



(33)



(34)



(35)

α-Amino acids with heterocycle side chains containing oxygen have also been synthesised. 1,3-Dipolar cycloadditon of nitrile oxide precursor (**33**) with 2-methylfuran gave the furoisoxazoline intermediate (**34**). This could be converted to four of the eight stereoisomers of L-(+)-furanomycin (**35**).[360] Progress in the synthesis of amino acids containing tetrahydrofuran- and tetrahydropyran amino acids has been reported[361] and 2,2-dimethyl-1,3-dioxane-4,6-dione derivatives of amino acids have been prepared by reaction of the appropriate amino acid with ethoxymethyleneisopropylidene malonate.[362]

Diastereoselective alkylation and/or protonation of chiral enolates have been

used to prepare enanteomerically pure azatyrosine, tribromo- and trichlorophenylalanine[363] and a series of phenylalanine derivatives containing halo-atoms on the benzene ring are reported.[364] Ninety new alkyl/arylsulfonyl and -sulfonylureido glycine hydroxamates have been synthesised and tested as inhibitors of *Clostridium histolyticum* collagenase.[365]

A range of novel heterocyclic substituted α-amino acids has been synthesised by cyclocondensation of (*S*)-2-tert-butoxycarbonylamino-4-oxo-hex-5-ylnoic acid tert-butyl ester with enamines, phenylhydrazine, hydroxylamine and Ph azide.[366]

The syntheses of 3-heteroaromatic-substituted alanines,[367] the optically active phenylglycine derivatives from *S*-(+)-*N*-(benzylidene)-*p*-toluenesulfinamide using Lewis acids and tert-amines,[368] new derivatives of *m*-nitrobenzoyl-D,L-asparagic acid, with potential fungitoxic activity, by the cyclisation of 2-(*m*-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazolin-5-ones have been reported,[369] and some *N*-mustards with esters of *N*-acyl-*m*′-aminobenzoyl-D,L-asparagic acid as supports by ring opening reactions of *N*-acyl-Δ^2-oxazolin-5-ones under the action of di-(β-chloroethyl)-amine[370] are reported.

A convergent synthesis of (*S*)-(−)-3-(2-carboxy-4-pyrrolyl)alanine from a commercially available dimethyl 1-aspartate in good overall yield has been reported,[371] and Fmoc-amino acid azides have been prepared from protected amino acids and NaN_3 by the mixed anhydride method. They are crystalline solids with a long shelf life.[372]

Progress towards the synthesis of fluorodihydroxyphenyl serine has been reviewed and the Evans aldol approach recommended.[373]

4.11 The Synthesis of α-Amino Acids Carrying Amino Groups and Related Nitrogen Functional Groups in Aliphatic Side Chains. – N^{α}-Substituted-N^{β}-protected hydrazinoglycinates have been readily prepared from hydrazines and bromoacetate esters, these materials being useful as potential monomers for solid phase synthesis of hydrazinopeptidoids,[374] and other new potential monomers for solid phase synthesis of hydrazinopeptoids, N^{α}-substituted-N^{β}-protected hydrazinoglycines and hydrazinoglycinals have been identified.[375]

A synthesis is reported of the N^{ω}-hydroxyiminoethyl derivatives of ornithine and lysine. The compounds were tested for inhibition of nitric oxide synthase inactivation[376] and all four *N*,*N*′-protected DAB sterioisomers, using an asymmetric Rh(I)-phosphine-catalysed hydrogenation of isomeric enamides as the key step, have been prepared.[377] An enantiospecific synthesis has been carried out of (*R*)-Boc-(Fmoc)-aminoglycine from (*S*)-Cbz-serine *via* the cyclic carbamate, (*S*)-4-Cbz-amino-2-oxazolidinone.[378]

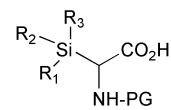
The syntheses of various diamino compounds, namely, (+)- and (−)-2,6-diaminopimelic acids,[379] (*S*,*S*)- and (*R*,*R*)-2-amino-3-methylaminobutanoic acid, from tert-butyl crotonate,[380] and differentially protected (2*S*,4*S*)-2,4-diaminoglutaric acids[381] have been reported. The differentially protected (2*S*,4*S*)-2,4-diaminoglutaric acids were synthesised for incorporation into peptides. Derivatives of N^{α}-amino-ω-isocyanato-, ω-ureido- and α,ω-diamino acids have been synthesised.[382]

4.12 Synthesis of α-Amino Acids Carrying Boron Functional Groups in Side Chains. – The synthesis of enantiomerically pure ω-borono-α-amino acids of various chain lengths using the general methodology involving the condensation of alkenyl and alkynyl bromides with Ni(ll) complex of the Schiff base derived from glycine and (*S*)-2-[*N*-(*N*-benzylprolyl)amino]benzophenone, and hydroboration of the intermediate ω-unsaturated α-amino acids with diisopinocamphylborane, and oxidation with acetaldehyde has been reported.[383] The synthesis of 4-borono-2-fluorophenylalanine, from 4-bromo-2-fluorotoluene[384] and *p*-boronophenylalanine, in six steps from 4-bromobenzaldehyde, has been reported.[385,386] Enantiomerically pure 4-borono-L-phenylalanine has also been synthesised.[387] Studies on the structure of the complex of the latter boron neutron capture drug, with fructose and related carbohydrates, using chemical and ^{13}C NMR methods have also been reported.[388]

4.13 Synthesis of α-Amino Acids Carrying Silicon Functional Groups in Side Chains. – The synthesis is reported of silicon- and germanium- containing α-amino acids and peptides. The synthesised compounds were used to compare C, Si and Ge bioisosterism[389] and β-trimethylsilyl- and -germylalanines have been prepared and studied by single crystal X-ray diffraction.[390]

The synthesis of allylsilane-containing amino acids *via* a Claisen rearrangement has been reported.[391] 3-Trimethylsilylalanine has been prepared enzymatically/microbiologically by two groups of workers.[392,393]

The first synthesis has been reported of α-trialkylsilyl amino acids (**36**, R = Et, CH_2Ph, $R^1 = R^2$ = Me, Et; R^3 = Me, Et, CMe_3, PG = Tos, Boc, Cbz).[394] The synthesis of silaproline, a new proline surrogate, has been reported.[395]



(36)

4.14 Synthesis of α-Amino Acids Carrying Phosphorus Functional Groups in Side Chains. – Readers looking for phosphorus analogues of amino acids should also look in this section.

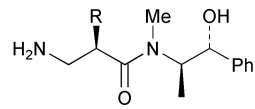
The synthesis is reported of *N*-alkyl-(α-aminoalkyl)phosphine oxides and phosphonic esters, *e.g.* $(MeO)_2P(O)CHPhNHCH(CH_2CHMe_2)CO_2CH_2Ph$ starting from α-amino acids.[396] The synthesis of protected analogues of phosphoserine and their incorporation into peptides has been reported[397] and 1-phosphaserine and 1-phosphaisoserine have been prepared using lipase SP 524. The four stereoisomeric intermediate hydroxyethyl phosphonic acids were separated by capillary electrophoresis with quinine carbamate as the chiral ion pair agent.[398]

A synthesis of the labelled iodinated inhibitor of aminopeptidase N, 2(*S*)-benzyl-3-[hydroxy(1′(*R*)-aminoethyl)phosphinyl]propanoyl-L-3-[^{125}I]-iodotyrosine[399] is reported. The syntheses of *N*-Fmoc-4-[(diethylphosphono)-2,2′difluoro-1′-hydroxyethyl]phenylalanine, as a phosphotyrosyl mimic for the prep-

aration of signal transduction inhibitory peptides,[400] phosphonic analogues of 4-hydroxyproline and 5-hydroxypipecolic acid,[401] and (*S*)-α-cyclopropyl-4-phosphonophenylglycine *via* a multi-step procedure starting from (*R*)-4-benzyloxyphenylglycine[402] have been described.

4.15 Synthesis of α-Amino Acids Carrying Sulfur-, Selenium- or Tellurium-containing Side Chains. – The synthesis has been reported of some Se- and Te-containing amino acids for use as probes for structural studies on proteins.[403] Optically pure amino acids, bearing side chain thioamides, have been synthesised by selective thiations on multiple-carbonyl containing substrates. The products are useful for solid phase peptide synthesis.[404]

Synthetic routes for the preparation of 2-chloroethylnitrososulfamide derivatives of amino acids, from chlorosulfonyl isocyanate *via* carbamoylation–sulfamoylation–cyclisation reactions,[405] and L-selenohomocysteine from L-selenomethionine.[406] The L-selenohomocysteine was used as a substrate for methionine synthase kinetic studies. The cysteine-derived amino alcohol (**37**) has been synthesised as a ligand for iridium(I)-catalysed asymmetric hydrogenation of unsymmetrical ketones.[407]



(37)

4.16 Synthesis of β-Amino Acids and Higher Homologous Amino Acids. – Reviews of diastereoselective approaches to the synthesis of γ-amino-β-hydroxy amino acids[408] and substitution by free radical and anionic chemistry in studies on γ-amino acids and γ-peptides[409] have been published, and a general strategy for the synthesis of the non-natural β^2-amino acids has been described.[410] Synthesis of ω-aminophosphonic acids have been reviewed.[411]

The asymmetric syntheses of β- and α-amino acids have been studied based on carbon radical addition to oxime ethers[412] and asymmetric acyl halide–aldehyde cyclocondensation reactions catalysed by Al(III) triamine complexes gave enantiomerically enriched β-*R*-substituted lactones which underwent ring-opening to give chiral β-amino acids.[413]

The synthesis of substituted β-amino acids has been reported. *N*-substituted β-alanines were prepared by the reaction of 3-amino-9-alkyl carbazoles with acrylic and itaconic acids[414] and *N*-quinolyl-β-alanines have been synthesised by reaction of aminoquinolines and acrylic, methacrylic and crotonic acids, and their biological activity has been investigated.[415] Catalytic enantioselective Mannich-type reactions of silyl enol ethers with aldimes have been performed using a novel chiral zirconium catalyst. The resulting β-amino acids were obtained with high yields and enantioselectivities[416] and the activation of Schiff bases by *N*-glycosylation has been shown to induce asymmetrical Mannich reactions with *O*-silyl ketene acetals to give β-amino acids.[417] β-Amino acids have also been prepared by addition of chiral enolates to nitrones *via* *N*-acyloxyiminium ions.[418]

The synthesis of α-substituted-β-amino acids *via* the amides (**38**, R = Me, Et, Pr, Allyl) has been reported, using pseudoephedrine as a chiral auxiliary,[419] and *via* the aza-aldol reaction of the chiral enolate derived from (2*S*)-*N*-propionyl-camphor sultam with *N*-diphenylphosphinyl imines.[420] β-Haloaryl-β-amino acid derivatives have been synthesised using a conjugate addition/oxidative deprotection strategy, employing lithium *N*-benzyl-*N*-α-methyl-4-methoxybenzylamide as a homochiral ammonia equivalent.[421]

α,β-Substitued β-amino acids have been synthesised using a diastereoselective alkylation by organocuprate reagents[422] and by the reaction of *N*-alkoxycarbonyl-1-methoxyamines with optically active 2-oxazolidinones[423] and β-substituted and β,β-disubstituted β-amino acids, which carry a hydroxyalkyl side chain, from sulfonimidoyl functionalised homoallylic alcohols.[424]

Synthetic routes have also been described for the substituted δ-amino acids. α,δ-Disubstituted-δ-amino acids were prepared by stereoselective alkylation of 5-substituted δ-lactams[425] and the first asymmetric synthesis of (*R*)-(−)-α-phenyl δ-amino valeric acid has been reported.[426] The synthesis is reported of 5-amino-4-hydroxy-2,6-dimethylheptanoic acid from *N*-Boc-L-valine methyl ester. The heptanoic acid is a hydroxyethylene isostere of Val-Ala dipeptide.[427]

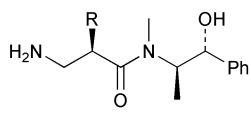
γ-Amino acids and γ-lactams have been prepared from nitro olefins and carboxylic acids using valine-derived 4-isopropyl-5,5-diphenyl-1,3-oxazolidin-2-one as an auxiliary for the enantioselective preparation[428] and *N*-methyl-γ-amino-β-hydroxy acids, essential components of several depsipeptides with interesting therapeutic profiles, have been synthesised *via* a totally stereocontrolled route of preparation.[429]

The synthesis of 'cyclic' amino acids has also been described. These are mainly of two types, the pyrrole-type, where the amino acid nitrogen is included in a ring structure, or the cycloalkane-type, where the amino group and carboxylic acid group are attached to a ring. An asymmetric synthesis of the cyclic β-amino acids generally (**39**, $n = 1$–3) has been reported.[430]

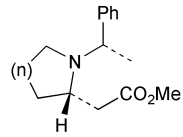
Syntheses of amino acids of the cycloalkane-type include those of cyclopropane and cyclobutane β-amino acids,[431] diastereo- and enantiomerically pure β-aminocyclopropanecarboxylic acids,[432] 2-aminocyclopentanecarboxylic acid and related alicyclic β-amino acids[433] and methyl (1*S*,2*R*)-1-amino-2,3dihydro-1*H*-indene-2-carboxylate, a new, constrained β-amino ester, using a novel tandem conjugate addition intramolecular electrophilic trap to construct the indane skeleton.[434] Amino acids, incorporating an amino cyclopropyl moiety, have been synthesised by a titanium-mediated transformation of *N*,*N*-dibenzyl-2-benzyloxyacetamide with a variety of alkylmagnesium bromides.[435]

Those of the pyrrole-type include all four stereoisomers of 4-hydroxypipecolic acid, from δ-amino-β-keto esters,[436] (−)-detoxinine, (**40**) the core unit of the detoxifying agent detoxin D_1, from an inexpensive starting material, L-ascorbic acid, *via* the key intermediate (**41**),[437] and a seven step synthesis for the preparation of *N*-benzyl-7-azaspiro[4.5]decane-1-carboxylates (**42**) from 2-oxocyclopentanecarboxylate. The latter are analogues of GABA.[438]

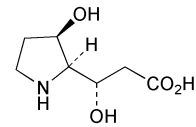
The preparation of an unusual amino acid that mimics a tripeptide β-strand and forms β-sheet-like hydrogen-bonded dimers by the condensation of suitably



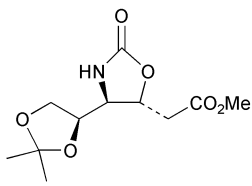
(38)



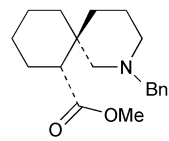
(39)



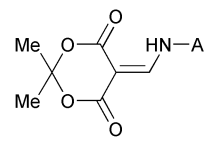
(40)



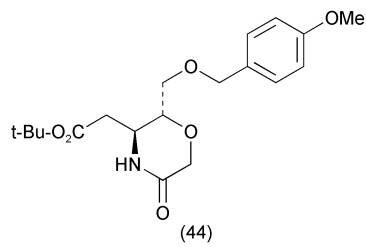
(41)



(42)



(43)



(44)

protected derivatives of hydrazine, 5-amino-2-methoxybenzoic acid and oxalic acid has been detailed.[439]

The syntheses of the methyl esters of the *N*-heteroamino-methylene malonic acids (**43**, A = 2-pyridyl, 2-(5-methylpyridyl), 2-pyrimidyl), which sublime to give oxopyidinopyrimidines,[440] 4-amino-3-(aminomethyl)benzoic acid, in three steps from 4-aminobenzoic acid[441] and *syn*-1-vinyl-2-amino alcohol derivatives by addition of (γ-alkoxyallyl)titaniums with chiral imines[442] have been reported.

Homologation of amino acids has been achieved *via* well-recognised mechanisms. The Arndt–Eistert approach has been used for the synthesis of Boc-/*Z*-/Fmoc-β-amino acids from *N*-protected α-amino acid fluorides in a two-step reaction[443] and *N*-Fmoc-L-β-homoglutamine and *N*-Fmoc-L-β-homoasparagine from *N*-Fmoc-L-α-glutamine and N^{α}-Fmoc-N^{γ}-trityl-L-asparagine.[444] The Michael addition of nucleophiles to *N*-acyl-*N*-(tert-butoxycarbonyl)dehydroalanine methyl ester has been used to synthesise β-alanines[445] and the same technique was used to produce the highly functionalised β-amino acid (3*R*,5*R*,6*R*)-3,6-diamino-5-hydroxyheptanoic acid, the key amino acid of sperabillins B and D,[446] and to synthesise the oxazinone (**44**), which can be alkylated to give protected *anti*,*anti* α-alkyl β-amino δ-hydroxy esters by Michael addition of the carbamoate moiety of the enoate (*R*,*E*)-$Me_3CO_2CCH{:}CHCH(CH_2OCH_2C_6H_4$-4-OMe)$CH_2O_2CNH_2$.[447]

α-Substituted-β-amino acid derivatives have been synthesised stereoselectively using Wolff rearrangement reactions[448] and the Wolff rearrangement of α-aminodiazoketones derived from N^{α}-urethane-protected α-amino acids that gives rise to the homologation of Fmoc-/Boc-*Z*-α-amino acids to β-amino acids

with concomitant formation of the corresponding pentafluorophenyl esters has been reported.[449]

The route of hydrolysis of heterocyclic rings has also been utilised. 2-Oxazolines have been prepared by $BF_3{\cdot}OEt_2$-catalysed regio- and stereo-selective oxirane ring opening of glycidic esters or amides with MeCN. The oxazolines were hydrolysed into β-amino-α-hydroxy esters or amides[450] and a variety of β-aminoalanine derivatives were prepared by regioselective cleavage of the C(3)–N bond of enantiomerically pure aziridine-2-methanols by nitrogen nucleophiles.[451]

Resin bound *N*-acylated amino acid aldehydes were converted in a single step to α-hydroxy phosphonates by a Pudovik reaction and in six steps to hydroxystatine amides, useful for constructing multiple aspartic acid transition state isosteres.[452]

4.17 Resolution of DL-Amino Acids. – The resolution of DL-amino acids is a key step in amino acid chemistry. This is usually achieved by preferential crystalisation, enzymically, *via* a chromatographic technique utilising a chiral recognition agent, by asymmetric transformation or by absorption onto a polymer/micelle substrate which possesses chiral recognition properties. Papers have been published reviewing the uses of aminoamidases in the enzymic resolution of amino acid amides (84 references)[453] and the separation of enantiomers by gas chromatography (168 references), where amino acids form one of the groups of chiral selector employed.[454]

Preferential crystallisation has been used for the resolution of D,L-α-alanine using L-alanine seed crystal. In this study the addition of OP surfactant was shown to accelerate the crystallisation of L-alanine.[455] The technique has also been used for the optical resolution of D,L-threonine by replacing crystalisation using L-alanine as an optically active co-solute[456] and the erythro- and threo-forms of 4-fluoroglutamic acid through their diastereomeric salts.[457] The phenomena of decrease in purity during the optical resolution of D,L-threonine by preferential crystallisation is discussed.[458]

Crystal structure–solubility relationships in the optical resolution of phenylglycine with (+)-10-camphorsulfonic acid have been studied in detail and the mechanism of the resolving ability discussed.[459]

Enzymic techniques have been used extensively. Enantiomerically enriched β-amino acids have been prepared by enzymic resolution.[460] The enantioselectivity of the lipase-catalysed hydrolysis of amino acid esters has been studied and found to depend on the source of the enzyme, the N-protecting group and the alcohol moiety of the ester[461] and the chiral discrimination of racemic carbazole carbonyl amino acids with linear alkyl side chains by bovine serum albumin was investigated by competitive replacement experiments using dansyl-L-proline and -D-norvaline as fluorescent probes; D-amino acids were bound to the L-proline site more strongly than the L-forms.[462]

Immobilised chymotrypsin on hydrophilic macroporous support has been used for the resolution of D,L-phenylalanine ethyl ester Schiff base. The L-isomer was hydrolysed and the D-isomer recovered unchanged to be hydrolysed chemi-

cally.[463] Horse-heart myoglobin has been shown to promote enantioselective hydrolysis of 4-nitrophenyl esters of amino acids, allowing nearly perfect kinetic resolution of the racemic *N*-Boc-phenylalanine ester (Boc-Phe-ONp)[464] and *N*,*N*-disubstituted α-amino acid phenolic esters have been resolved enzymatically using pig liver esterase on the multi-gram scale and the configuration confirmed by X-ray analysis.[465]

Optically active *N*-benzoyl amino acids have been obtained by the dynamic kinetic resolution of racemic 2-benzyl-4-substituted-5(4*H*)-oxazolones in the presence of an alcohol using *Candida antarctica* lipase B as a catalyst[466] and lipase PS has been used to resolve *cis*-β-hydroxypipecolic acids.[467]

Penicillin G acylase catalysed the acylation of the L-isomers of methyl esters of phenylglycine and derivatives. The process allows the isolation of the enantiomerically pure D-phenylglycine, suitable for conversion into β-lactam antibiotics,[468] and the pure diastereoisomers of 4-fluoroglutamine and 4-fluoroisoglutamine where prepared from the corresponding 4-fluoroglutamic acids. Glutamic decarboxylase treatment of the acids leads to chiral 2-fluoroGABA.[469] A technique for the resolution of *N*-acetyl-D,L methionine methyl ester by protease-catalysed hydrolysis with a mild base as the control agency has been described.[470] Various chromatographic techniques have been used to resolve D,L amino acids and their derivatives. The resolution of basic D,L-amino acids has been effected by direct thin layer chromatography, using a pharmaceutical industrial waste as a chiral impregnating agent,[471] and normal phase TLC has also been used to resolve dansyl-D,L- amino acids on plates impregnated with vancomycin.[472] The resolution of dansyl amino acids, using β-cyclodextrin as a mobile phase additive in reversed-phase TLC, has also been reported, and the effect of structure on the resolution has been studied.[473]

A new π-basic chiral stationary phase has been proposed for the separation of amino acid enantiomers by liquid chromatography. The stationary phase proved especially useful for separating π-acidic *N*-(3,5-dinitrobenzyl)-α-amino amides and esters.[474] HPLC has been utilised to resolve unusual α-amino acids, using direct (Crownpak or Chirobiotic T) stationary phase and indirect methods (precolumn derivatisation)[475] and (1*S*,2*S*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propylisothiocyanate has been proposed as a new chiral derivatising agent for the HPLC separation of amino acids with two chiral centres.[476] The same group, working with β-substituted tryptophan derivatives, separated all four diastereoisomers by direct (teichoplanin bonded or cyclodextrin bonded stationary phases) or indirect (precolumn derivatisation by chiral reagents) methods. The indirect methods proved more effective.[477] β-Heterocyclic and β-naphthylalanine and phenylalanines were separated by reversed phase HPLC after derivatisation by 1-fluoro-2,4-nitrophenyl-L-valinamide. All L-isomers were eluted faster.[478]

Protonated complexes of amino acids with β-cyclodextrin, produced in the gas phase by electrospray ionisation, were shown to undergo exchange of the amino acid with *N*-propylamine. The rate of exchange varies with the chirality of the amino acid; the enantiomeric excess can thus be determined[479] and copper(II)-assisted enantiomeric analysis of D,L-amino acids using the kinetic method has been studied and the chiral recognition and quantification in the gas phase has

been reported.[480]

Column chromatography (silica gel column) has been used for the resolution of racemic amino acids, using *N*-substituted 2-amino-4-pentenoic acids as a protecting group[481] and the temperature-dependence of the elution order of cyclic α-amino acid enantiomers on copper(II)-*N*,*S*-dioctyl-D-penicillamine ligand exchange column has been studied thermodynamically and a mechanism postulated for the separation.[482] Capillary electrophoresis has been used to separate underivatised amino acids, using copper(II):(*S*)-3-aminopyrrolidine:L-histidine ternary complex as a chiral selector,[483] and the chiral determination of amino acids by capillary electrophoresis and laser-induced fluorescence at picomolar concentrations has been reported.[484]

A study has been reported of the entiomeric separation of *N*-Fmoc amino acids by capiliary electrokinetic chromatography using sulfobutyl ether-β-cyclodextrin as a chiral additive,[485] and chiral analysis has been performed on amino acids in biological solutions by micellar electrokinetic chromatography with laser induced fluorescence detection.[486]

The separation of enantiomers by preparative capillary isotachophoresis, using 2,4-dinitrophenyl-D,L-norleucine as a model analyte has been reported.[487]

Several groups of workers have employed asymmetric transformation as a means of achieving resolution often followed by chromatographic separation. Indirect chiral separation methods based on enantiomeric derivatisations have been developed to monitor optical purity of uncoded amino acids and new series of amino acids using Marfey's reagent for the amino group and (1*R*,2*R*)- or (1*S*,2*S*)-2-amino-1-(nitrophenyl)-1,3-propanediol reagents for the carboxyl group. The diastereomeric derivatives were separated using RP-HPLC and NP-HPLC[488] and homocysteine has been resolved by derivatisation with 4-aminosulfonyl-7-fluoro-2,1,3-benoxadiazole followed by capilliary electrophoresis with γ-cyclodextrin.[489] The validity of the three point interaction model has been examined in the guest exchange reaction involving cyclodextrins and amino acids, and a mechanism for the exchange has been proposed.[490] 4-Hydroxyphenyl- and 4-fluorophenylglycine have been resolved using phenylglycine and (+)-10-camphorsulfonic acid.[491]

A dynamic kinetic resolution of *N*-phthalyl amino acids by stereoselective esterification has been examined using (*S*)-α-methylpantolactone as the chiral auxilliary[492] and the resolution of 1-(2-furyl)-2,2-dimethylpropylamine, an intermediate on a synthetic route to tert-leucine, followed by oxidation, was shown to provide a useful route to (*R*)-and (*S*)-tert-leucine.[493]

The use of polymers and micellular systems to achieve resolution has been reported. A highly enantioselective polymer, imprinted with an organophosphorus compound, was useful for the separation of tryptophan methyl esters[494] and a cross-linked polyvinyl alcohol membrane with L-proline as a chiral ligand has been used for the resolution of amino acids. L-Isomers permeated predominantly through the membrane.[495] The use of ultrafiltration of enantioselective micelles has been shown to provide a low energy, scalable process for the preparation of enantiomerically pure compounds. A model involving the complexation of phenylalanine enantiomers by cholesteryl-L-glutamate anchored in

non-ionic micelles of nonyl-Ph-polyoxyethylene [E10] ether has been reported[496] and a large-scale process for the separation of amino acid enantiomers has been reported in which copper(II)-amino acid derivatives dissolved in non-ionic surfactant micelles were used as the chiral selectors.[497]

A pair of artificial enantiomeric receptors composed of (*S*,*S*)- or (*R*,*R*)-chiral bicyclic guanidinium azacrown ether and (tert-butyl diphenylsilyloxy)methyl group for amino acid zwitterions selectively recognised either L- and D- amino acids[498] and the use of zinc bilinone (the chiral helical dimer of the zinc complex of linear tetrapyrrole) as a chiral recognition agent for α-amino esters is reported.[499]

5 Physico-chemical Studies of Amino Acids

5.1 X-Ray Crystal Analysis of Amino Acids and Their Derivatives. – Crystal structure analysis data have been reported for the following amino acids: D,L-cysteine,[500] D,L-isoleucine and D,L-alloisoleucine,[501] L-arginine phosphate monohydrate,[502, 503] D,L-arginine monohydrate at 100°,[504] L-arginine fluoroborate,[505] ammonium- and methylammonium *N*-acetyl-L-threoninate,[506] *N*-acetyl-L-phenylalanine,[507] sarcosinium trifluoracetate – the N-C-COOH of the protonated molecule is almost completely planar,[508] *N*-methyl-D,L-aspartic acid monohydrate,[509] *N*-methyl-D,L-glutamic acid,[510] L-histidinium dihydrogenarsenate orthoarsenic acid,[511] *N*-benzoylphenylalanine (also solid state ^{13}C NMR),[512] *N*-acetyl-β-trifluromethyl tryptophan ethyl acetate,[513] *N*,*N*-bis(*N*-methylsuccimido) β-alanine, (shows photochromism in its europium-1,10-phenanthroline complex),[514] complexes of maleic acid with L-histidine and L-lysine,[515] the phenylalanine complex of molybdenum-θ-allyl-$(CO)_2$,[516] and other highly derivatised amino acids: *N*-(6-amino-3,4-dihydro-3-methyl-5-nitroso-4-oxopyrimidin-2-yl) derivatives of glycine, valine, serine, threonine and methionine,[517] racemic N^{α}-(-t-butyloxycarbonyl)-L-phenylalanine *N*-methoxy-*N*-methylamide,[518] *N*,*N*-bis(8-hydroxy-5-quinolinemethyl)glycine ethyl ester,[519] 2-methyl-*N*-[(2-nitrophenyl)sulfonyl]alanine and 1-[(2-nitrophenylsulfonyl)-amino]cyclohexanecarboxylic acid.[520] Two conformationally-restricted 4,5-dihydroxynorvaline analogues with a norbornane skeleton,[521] 2,3,5,6,7,8-hexahydro-3-(1-methyl-2-oxopropyl)-6,8-methano-7,7,8a-trimethyl-5*H*-1,4-benzoazin-2-one and its 1-hydroxy derivative,[522] and C-terminal amidated amino acid hydrochlorides.[523]

5.2 Nuclear Magnetic Resonance Spectrometry. – The protonation states of a series of conformationally constrained amino acids (piperidine carboxylic acids) have been studied and correlated with theoretical results from HF/6-31 + G* calculations.[524]

The band shape analysis of delayed slow-passage optically detected magnetic resonance has been reported for the photoexcited triplet state of tryptophan.[525] The absolute configuration and enantiomeric analysis of amines and amino acids has been determined using non-chiral derivatising agents and deuterium NMR,[526] conformational equilibrium and intramolecular hydrogen bonding in

nipecotic acid derivatives has been investigated.[527] Solid state NMR has been reported of amino acids and peptides.[528] Caution should be exercised during determination of the absolute configuration of chiral amines by NMR using MPA derivatisation and Ba^{2+} complexation; chemical shifts show inconsistencies with the proposed model relating them to absolute configurations.[529] The characteristics have been determined of the intramolecular H bond in β-alanine, proline, threonine and cysteine by PMR.[530] The rotational isomerism about the C(2)–C(3) bond in aspartic acid and its phosphonic analogues have been studied by PMR. The characteristic vicinal coupling constants were dependent on the populations of the rotamers.[531]

Enantiomeric discrimination in the NMR spectra of underivatised amino acids and α-methyl amino acids has been observed using (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid as a chiral discriminating agent.[532,533] Samarium(III):(*R*)- or (*S*)-propylenediamine tetraacetate complex has been shown to be useful as a water-soluble chiral shift reagent for use in high-field NMR.[534]

Theoretical studies of the ^{13}C NMR of amino acids has been reviewed.[535]

Both the carboxyl and the hydroxyl ^{17}O resonances of the carboxylic acid group in a tyrosine derivative have been observed for the first time by ^{17}O NMR.[536]

Iodine-127 NQR, IR and X-ray analysis of α- and β-alanine and L-lysine have been reported.[537] NMR studies are reported of the Pt(II) and Pd(II) complexes of glycine[538] and bisalaninates.[539]

Multidimensional variants of the dipolar exchange assisted recoupling (DEAR) NMR have been applied to determinations of ^{13}C-^{14}N dipolar local field spectra in amino acids and peptides.[540] Changes occurring during complexation of praseodymium with serine have been monitored by NMR using spin density matrices.[541]

5.3 Optical Rotatory Dispersion and Circular Dichroism. – The use of optical rotation, CD and other chiroptical properties for the determination of absolute configuration of natural products has been reviewed.[542] The absolute configurations of α-phthalimido carboxylic acid derivatives have been determined from CD spectra.[543] A theoretical treatment has been reported of the photoelectron spectra and CD of L-alanine[544]

5.4 Mass Spectrometry. – Mass spectrometry applied routinely to assist in the structural investigation of amino acids has largely been omitted from this section.

The mobilities of twenty common amino acids have been determined by electrospray ionisation ion mobility spectrometry; not all mixtures of amino acids could be separated by this technique.[545] The fragmentation mechanisms of α-amino acids, protonated under electrospray ionisation, have been the subject of a collisional activation MS and *ab initio* theoretical study.[546] Eight amino acids have been analysed by matrix-assisted laser desorption/ionisation time of flight mass spectrometry and electron-ionisation techniques.[547] Arginine has been shown to form protonated clusters when examined by electrospray ionisation.

This phenomenon has been studied by tandem mass spectrometry.[548] The mass resolved electronic spectrum of cold tryptophan molecules has been obtained by a novel desorption method as a vapourisation source coupled with a supersonic expansion.[549]

The chiral recognition of nineteen common amino acids has been achieved from the collision-induced dissociation spectra of protonated trimers formed from the electrospray ionisation of amino acids in the presence of chiral selectors such as *N*-(tert-butoxycarbonyl)phenylalanine.[550]

Enantiomeric excess of amino acids has been determined by collision-induced dissociation spectra of protonated trimers formed by electrospray ionisation in the presence of a chiral selector.[551] Matrix assisted laser desorption/ionisation mass spectrometry has been used to differentiate isotopically labelled (pseudo-enantiomeric) amino acids using cyclodextrin as a host.[552]

The structures of the fragmentation products of the complex of glycine with zinc(II), produced by electrospray ionisation, have been studied.[553,554]

EI and CI mass spectra of *N*-dinitrophenyl derivatives of amino acids using a particle beam interface show characteristic fragmentation patterns, useful for identifying the amino acids.[555]

5.5 Other Spectroscopic Studies of Amino Acids. – This section covers the other common spectroscopic techniques, which have expanded to cover applications such as conformation determination, in many cases these have been combined with theoretical studies. Theoretical and experimental studies of the vibrational spectra (IR and Raman) of *N*-acetyl-L-alanine[556] and L-valine and L-leucine nitrate[557] have been reported. The IR and molecular structure of zwitterionic L-β-phenylalanine have been determined and compared with the results from *ab initio* calculations.[558]

Conformational studies have included the UV and IR of each of the seven conformational isomers of tryptamine,[559] while gas phase IR and UV ion dip spectroscopy of phenyl alanine has been used to study the most stable conformers.[560] The polymorphic transition of D,L-norleucine from the α-form to the γ-form has been investigated using temperature-scanning time-resolved FTIR.[561] The zwitterions of L-alanine were studied by IR spectra in a KBr matrix, together with the vibrational absorption and vibrational CD spectra. Theoretical calculations were also performed.[562,563] Amino acid salts have also been measured; the IR of sodium and calcium salts of α-amino fatty acids,[564] the FTIR and FT-Raman spectroscopy of D,L-homocysteine and its complexes with Na, K and Ca ions,[565] while in the IR of the monodeuterated salts of tyrosine, valine and some peptides, irradiation in the spectral region produces spectral holes and antiholes resulting from rotation of CD-containing moieties.[566]

Other complexes of amino acids have been studied by IR; the IR and Raman spectra are reported of Cu(II) complexes of aspartic and glutamic acids. The spectra are discussed in relation to their crystal structures.[567] Raman spectra of L-threonine[568] and L-alanine[569] crystals under pressure showed that both underwent a pressure induced phase transition.

The effect of reducing the temperature on the IR spectra of *N*-(tert-butoxycar-

bonyl)amino acids has been reported.[570] The structural changes of amino acids implanted with low energy ions have been studied by FTIR.[571, 572]

Various fluoro-organic compounds, including fluoro-amino acids derivatives were identified in pure and mixed samples by Raman and fluorescence spectra.[573] The colourimetric determination of aromatic amino acids by reaction with 4-chloro-7-nitro-2,1,3-benoxadiazole by measuring absorption maxima at 440–462 nm has been reported.[574]

Analysis has been reported of particle beam-hollow cathode glow discharge atomic emission spectrometry of aromatic amino acids and organomercury and lead compounds,[575] as has the emission spectroscopy of α,ω-diamino acids whose ω-amino group is coupled to a luminescent ruthenium fragment. The α-amino group was protonated. Effect of length of side chain on excited state decay rates has been studied.[576]

X-ray absorption spectra of selenocysteine, selenocystine and sulfo-selenocystine have been compared with the corresponding sulfur K-edge spectra.[577]

Square-wave adsorptive stripping voltammetry has been applied to the study of the interaction of cysteine with monosaccharides at physiological pH. The study was optimised with respect to accumulation time, accumulation potential, scan rate and drop size.[578]

Binding mechanisms and solvent effects have been studied for the molecular recognition of amino acids with zinc porphyrin receptors carrying twelve ester groups.[579]

5.6 Physico-chemical Studies of Amino Acids. – The sub-sections in this chapter have continued with the addition of a new section for measurements of underivatised amino acids in the gas phase.

5.6.1 Measurements for Amino Acid Solutions. Studies of solutions of familiar α-amino acids have lead to the determinations of apparent molar volumes,[580–582] partial molar volumes,[583–586] standard molar enthalpies of solution[587–589] and dilution,[590–594] enthalpies of dissociation,[595, 596] mixing,[597] and protonation.[598] Other properties measured have been viscosity,[599] densities,[600] conductivities,[601–603] solubilities,[604, 605] polarisability, refractive index, solubility and pH and other properties were determined on aqueous L-arginine solutions,[606] dissociation constants,[607, 608] and diffusion coefficients[609]

The effects of amino acids on the crystallisation of other materials hydroxyapatite,[610, 611] calcium phosphate[612] and calcium carbonate[613] have been studied, as well as the crystallisation of some amino acids (metastable crystalline phase of L-glutamic acid (α-form))[614] and single crystals of L-arginine phosphate monohydrate.[615] A study is reported of the crystallisation of glycine and phenylalanine in water-isooctane-AOT microemulsions.[616]

Studies of the solubilities of amino acids with nitrate salts continue; with sodium and potassium,[617] and zinc with histidine, methionine or phenylalanine.[618] Isopiestic studies have been reported on the systems $\{NaCl + BaCl_2 + mannitol_{(sat)}(aq)\}$ and $\{KCl + glycine + mannitol_{(sat)}(aq)\}$ at 298.15 K.[619]

Further studies on the gel forming properties of amino acids derivatives have

continued, aqueous gel-like solutions of *N*-acyl-aspartic acids (dodecanoyl–octadecanoyl) formed fibrous supramolecular assemblies which were investigated by atomic force microscopy, small angle neutron scattering and small angle X-ray scattering. The fibres are laterally organised,[620] while aroyl L-cystine derivatives were effective at gelating water.[621] The surfactant properties of different types of derivatives of glutamic acid have been reported.[622]

The effect of cationic surfactants (CTAB and CPB) on the addition–elimination type interaction between aspartic acid and ninhydrin is to increase the peudo first order rate constant.[623]

The characteristics of amino acid extraction from NaCl solutions by reverse micelle using ammonium bis(2-ethylhexyl) phosphate as a surfactant have been reported.[624] A proton transfer reaction, occurring during the extraction of amino acids has been studied using extraction of tryptophan with di(2-ethylhexyl)hydrogen phosphate (D2EHPA) in n-octane and n-octane/n-octanol. In octane, both 1:1 and 1:2 complexes were formed which tended to form clusters. No cluster formation was seen in the more polar solvent system.[625]

Overall partition coefficients of the acid and amine components of amino acid derivatives in an aqueous/organic biphasic system were studied experimentally and theoretically. Partition equilibrium and pH change after partition were predicted by the model.[626]

Studies have been performed on the zwitterions of glycine,[627, 628] *N*-acetylcysteine,[629] aspartic acid[630] and γ-aminobutyric acid.[631]

The kinetics and mechanism of the protonation reactions of amino acids, both inter- and intramolecular, have appeared in several studies; protonation constants,[632, 633] protonation equilibria of L-ornithine and L-glutamic acid in aqueous DMF,[634] the mechanism of interconversion between neutral and zwitterionic forms of glycine has been studied theoretically; proton transfer *via* a water bridge is proposed.[635] The mechanism and energetics of the intramolecular proton transfer of serine in aqueous solution have been reported.[636]

Mechanism of proton transfer from neutral to zwitterionic form of amino acids has been studied.[637] The third order rate constants for the general base-catalysed reaction between *N*-chlorotaurine and its protonated form and for general acid catalysis of the reverse process have been determined. A mechanism for the reaction is thought to involve *N*,*N*-dichlorotaurine as an intermediate.[638]

Proton exchange rates in *N*-acetylglycine have been determined.[639] Formation and stability of the enolates of glycine and its derivatives have been studied. Second order rate constants were measured for carbon deprotonation of the glycine zwitterion, *N*-protonated glycine methyl ester, betaine methyl ester and betaine by D_2O.[640] Solution studies of complexes of amino acids with metal cations have also been reported.

The structure and stability of amino acid phosphonic acid–metal complexes have been reviewed.[641] Complexes can be divided into binary-amino acid only with metal; with copper,[642–646] with chromium(II),[647, 648] cadmium,[649] vanadium (IV),[650] zinc,[651, 652] and d-block metals.[653]

Ternary complexes contain an amino acid unit and a secondary ligand; the transition metals with an amino acid and γ-picoline,[654, 655] β-picoline and manga-

nese,[656] quadridentate ligands from haloacetylated amino acids and bis(picolyl)-amine then reacted to form trigonal bipyramidal complexes with zinc. The crystal structure of one complex is reported.[657] Complexes have also been studied of amino acids with imidazoles,[658, 659] with sulfamethoxypyridazine,[660] with 2,2-bipyridine[661] and complexation of *N*-(2-nitrophenylsulfonyl)glycine with metals(II) with and without 2,2′-bipyridine in aqueous solution to identify the type, number and stability of the complex species as a function of pH and metal-to-ligand ratio.[662]

Chiral complexes of substituted η^3-butadienyl molybdenum complexes, prepared by reaction of a chlorocarbonyl compound with amino acid esters, were investigated by NMR. Compounds containing one or two stereogenic centres gave rise to dimeric complexes containing dibutadienyl bridging ligands.[663]

Complexes of lanthanum[664, 665] and europium(III)[666] with amino acids and other ligands have also been studied. Paper electrophoresis has been used to study the complexation of dioxouranium with serine and valine. The results suggested complexation through the carboxylate group of the zwitterion.[667]

Other mixed complexes studied have contained proline or 1-hydroxyproline, Cu(II) and an amino acid enantiomer[668] and cystine in the presence of cadmium and folic acid. Adducts are fomed between cystine thiolate and folic acid.[669]

The differential hydration properties of hydrophobic groups of a homologous series of α,ω-amino carboxylic acids were measured in H_2O and D_2O.[670] Time-of-flight neutron diffraction measurements have been carried out on alkaline aqueous 2 mol% glycine solution in heavy water. The hydrogen bonds formed by the amino group nitrogen differ significantly from those formed in neutral solution.[671] A study has been reported of the effects of circulation and facilitated electromigration of amino acids in electrodialysis with ion exchange membranes.[672] An investigation has been reported of the dependence on solvents of optical absorption and emission of a complex of bacteriochlorophyll a with serine.[673]

A voltammetric study is reported of amino acids on gold, platinum, copper and nickel electrodes.[674]

5.6.2 Measurements for Solid Amino Acids. Enthalpies of combustion and formation of eleven aliphatic amino acids[675] and enthalpies of formation crystalline D,L-valine[676] have been reported.

The piezoelectric, dielectric and pyroelectric properties of the twenty protein amino acids have been reported.[677] Surface polarity of α-amino acid crystals has been studied using solvatochromic dyes and compared to poly(amino acids) with the same side chain,[678] and electrostatic properties of α-glycine measured.[679]

Phase transitions have been observed in crystals of D- and L-alanine and valine,[680] and L-alanine.[681] Knoop microhardness anisotropy on the cleavage plane of single crystals of L-arginine hydrochloride monohydrate and the corresponding hydrobromide has been reported.[682] Dislocation resonance damping in L-arginine phosphate monohydrate single crystal has been observed using longitudinal ultrasonic attenuation and velocity measurements.[683, 684] Crystals of (*S*)- and racemic-*N*-benzoylalanine methyl ester had different melting points, reflect-

ing differences in lattice energy.[685] A study has been reported of the refinement and purification of crude glutamine crystal.[686]

5.6.3 Amino Acid Adsorption and Transport Phenomena. Partition of amino acids between immiscible organic and aqueous phases continues to interest researchers; ammonium bis(2-ethylhexyl) phosphate has been used as a reverse micelle surfactant for extracting amino acids from highly concentrated NaCl solutions,[687] the effect of pH on amino acid extraction ratios using AOT reverse micelle,[688] and forward and backward extraction rates of phenylalanine in reversed micellar extraction measured.[689] Extraction and concentration of L-phenylalanine from aqueous solution containing L-phenylalanine has been performed with and without L-aspartate using emulsion liquid membrane.[690] The equilibrium and kinetics have been studied of the extraction of glycine from HCl solutions by reversed micelles[691] and the extraction of amino acids with emulsion liquid membranes using industrial surfactants and lecithin as stabilisers.[692] A new mechanism is proposed for the extraction of amino acids from water to organic solvent using di(2-ethylhexyl)phosphoric acid. The mechanism explains the dependence of the equilibrium constant on the loading ratio.[693] A novel artificial receptor for aromatic amino acid zwitterions, prepared in three steps from a chiral bis(aminomethyl)bicyclic guanidinium salt, allows the aromatic amino acid to move from aqueous solution to DCM.[694] Extraction of amino acids from aqueous solutions into chloroform occurs using di(2-ethylhexyl)phosphoric acid in the presence of dicyclohexyl-18-crown-6.[695] Spectrophotometry and pH measurements have been used to study the extraction of Co(III) and Cu(II) complexes of amino acids from an aqueous donating phase into chloroform liquid membrane containing calix[4]resorcinarene; Cu(II) complexes are extracted more efficiently, especially if the aqueous phase is alkaline.[696] A tryptophan–tyrosine mixture has been separated by non-ion exchange sorption on an anion exchanger with hydrochloric acid. The sorption of the amino acids was temperature dependent.[697]

Studies have been reported of the adsorption of amino acids onto surfaces; onto silk fibroin, and synthetic polypeptides[698] and films; grafting of amino acids onto PET film surface was found to improve the surface properties of the amino acids such as wetability and neg. ion activity for use in medical techniques.[699] Overoxidised polypyrrole films templated with L-glutamate selectively take L-glutamic acid and other L-amino acids into the film.[700]

The thermodynamic functions for the sorption of aromatic amino acids on KU-2x8 sulfocationite in the H-form have been determined.[701] A study is reported of the adsorption and electroadsorption of amino acids from aqueous solution on uncharged and electrochemically polarised carbonaceous material.[702] Adsorption of tyrosine on to activated carbon/water interface has been shown to be pH dependent.[703] Adsorption of glycine and alanine on montmorillonite with or without divalent cations has also been studied.[704]

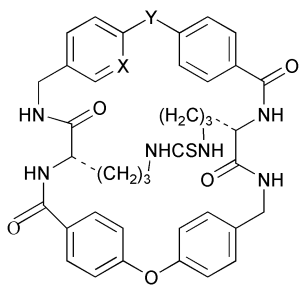
Studies of amino acids adsorbed onto metal surfaces have also been published; the microscopic monolayers of cystine and cysteine assembled on Au(111) form hydrogen bonded cluster networks,[705] adsorption of L-cysteine on gold by elec-

trochemical desorption and copper(II) ion complexation has been studied.[706] A combined density functional theory and X-ray emission spectra study has been reported of the electronic structure and surface chemistry of glycine adsorbed on Cu(110).[707] The adsorption behaviour of aspartic acid on Cu(001), studied by scanning tunnelling microscopy, shows features such as inability to form ordered structures which are different from the adsorption behaviour of other amino acids.[708] The adsorption behaviour of amino acids on a stainless steel surface has been studied.[709]

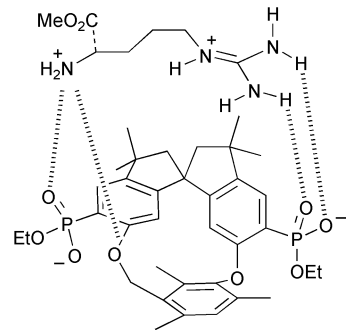
5.6.4 Host–Guest Studies with Amino Acids. Studies on the complexation of tryptophan and its derivatives with cyclodextrins continue. The 1:1 host guest complexes formed by 6α-(2-amino-ethylamino)-6α-deoxy-β-cyclodextrin and (*R*)- and (*S*)-tryptophan have been studied by pH titrimetric and NMR spectroscopic studies.[710] Organoselenium-containing β-cyclodextrins and their complexes with L- and D-tryptophan were studied by NMR, IR and combustion analyses.[711] Other cyclodextrin studies have appeared; a correlation has been found between the conformation and chiral recognition of a series of amino acid complexes with β- and γ-cyclodextrins using titration microcalorimetry and PMR,[712] and the inclusion complexation behaviour of the methionine, proline and isoleucine derivatives of β-cyclodextrin has been studied by fluorescence spectrometry. The amino acid derivative showed increased binding ability with 8-anilino-1-naphthalenesulfonic acid ammonium salt compared to the parent cyclodextrin, but decreased ability with Rhodamine B.[713] Enantioselectivity towards amino acids by metallo-6^A-deoxy-6^A-hydroxyethylamino-β-cyclodextrin has been investigated by potentiometric titration of the amino acids with NaOH. Nickel complexes show the greatest enantioselectivity.[714] A thermodynamic study is reported of the complexation of γ-cyclodextrin with *N*-carbobenzyloxy aromatic amino acids and ω-phenylalkanoic acids.[715]

The stability constants for the inclusion complexes of *p*-sulfonatocalix[4] arene with amino acids have been measured.[716] The complex of *p*-sulfonatocalix[4]arene with L-lysine shows a cationic substrate spanning the hydrophobic bilayer.[717] Chiral homoazacalixarenes possessing amino acid residues have been prepared. Their preferred conformation was a cone.[718] The rate of alcoholysis of *N*-acetyl-1-amino acids in methanol increased markedly in the presence of *p*-sulfonatocalix[*n*]arenes compared to *p*-hydroxybenzenesulfonic acid; NMR indicated the formation of an inclusion complex between the calixarene and *N*-acetyl-1-histidine.[719] Cryptand[222] undergoes selective complex formation with some polar and aromatic amino acids; thermodynamic functions and equilibrium constants of complex formation were calculated for histidine, threonine and glutamine.[720] Liquid–liquid extraction of non-protein amino acids by 18-crown-6 and cryptand[2.2.2.] shows a relationship with the amino acid structure.[721] Two new receptors (**45**, X = N, Y = O; X = CH, Y = CH_2) have been prepared. The presence of the pyridyl unit provides an additional H-bonding functionality.[722] A new class of C1- or C-2 symmetrical host molecules based on a spirobisindane skeleton has been used for diamines. One host molecule prefers short rigid diamines (lysine), the other longer α,ω-dications

(**46**).[723] Molecular recognition of amino acid esters by 5-(2-carboxyphenyl)-10,15,20-triphenylporphyrinatozinc(II) was investigated by UV–vis spectrophotometric titration method. The host–guest binding mode was studied by PMR.[724] A further study on fixed site heteropolysiloxane membranes containing grafted macrocylic receptors, used to separate mixtures of amino acids, has been reported. A dual transport mechanism is proposed.[725]



(45)



(46)

A liquid chromatography and ultrafiltration study has been reported of the binding of D- and L-tryptophan to bovine serum albumin in the pH range 7 to 11.[726]

5.6.5 Gas Phase Measurements. Studies of cationised glycine and its derivatives in the gas phase have been reported; $Gly.M^{2+}$ (M = Be, Mg, Ca, Sr, Ba) – the divalent metal ions dramatically influence the structure of glycine in the gas phase.[727] The influence of derivatisation, proton affinity and alkali metal addition on the stability of a series of N- and C-methylated glycines cationised by alkali ions[728] and the enol of glycine $H_2N\text{-}CH=C(OH)_2$ generated in the gas phase by neutralisation of the corresponding radical cation has been studied. The reionisation shows that the enol exists and does not isomerise significantly to the more stable glycine.[729]

5.7 Molecular Orbital Calculations for Amino Acids. – For a large range of amino acids, the following properties have been studied; the mechanism of proton exchange between amino acids side chains and water,[730] VAED characterisation (Vector of atomic electronegative distance) and ^{13}C simulation for 20 natural amino acids using MATLAB and True basic programs,[731] solvation free energies (hydrophobicities),[732] selected properties of amino acids have correlated using a variable connectivity index, $^{1}\chi^{f}$, which is obtained by introducing variable weights into a generalised connectivtiy index.[733]

Glycine has featured most heavily in MO calculations with the following; the radiation products of glycine crystals, structures are proposed for the four radicals formed,[734] the potential energy surface of glycine, and the vibrational state and spectroscopy computed from the results,[735] solvent effects on intra-

molecular proton transfer in glycine hydrated by three water molecules,[736] the effect of ionisation on the relative stabilities of the four lowest conformers of glycine and the intramolecular proton transfer process transfer in glycine radical cation,[737] the structure and energetics for the four lowest energy conformers of glycine,[738] the mechanism of fragmentation of protonated glycine in the gas phase,[739] the lattice energies of the three polymorphs of glycine,[740] solvent effects on the energetics and molecular response properties of glycine and alanine[741] mechanism of the mass spectral fragmentation of protonated glycine at low energy,[742] a new solvation model combining discrete and continuous descriptions of the solvent has been applied to the relative stabilities of the neutral and zwitterionic forms of glycine,[743] and the interconversion barriers of glycine and L-alanine conformers.[744]

Other amino acid studies have comprised side chain conformational analysis on two derivatives of asparagine and asparaginamide in their γ_1-backbone conformation,[745] charge density, dipole moment, electrostatic potential and electric field gradients for L-asparagine monohydrate,[746] gaseous neutral and zwitterionic forms of alanine show parity-violation,[747, 748] the correlation time for the reorientation of the methyl side chain in crystalline L-alanine,[749] conformational behaviour of β-alanine zwitterion in aqueous solution,[750] phosphorylation and dephosphorylation of serine, threonine and tyrosine phosphate,[751] proton affinities and gas-phase basicities of glycine, serine and cysteine.[752]

Complexation of cations with amino acids in the gas phase have also been studied; interaction of neutral and zwitterionic glycine in the gas phase with Zn^{2+} ions,[753] zwitterionic glycine bridged with NaCl,[754] cation-σ interactions for complexes of Na^+ and K^+ with aromatic amino acids,[755] gas phase metal ion (Li^+, Na^+, Cu^+) affinities of glycine and alanine,[756] a conformation and hydrogen bonding study of the complex of alaninamide and water. The lowest energy conformer had a network of intermolecular hydrogen bonds from the amide to water and from the water to the carbonyl oxygen.[757]

An *ab initio* analysis has been reported of the stability of different conformers of glycine, *N*-methylglycine and *N*,*N*-dimethylglycine. The effects of solvent and group size on the tautomerisation were studied.[758] The same authors have also reported similar calculations for fluoroglycine and have compared the two sets of results.[759, 760]

Derivatives of amino acids studied have included: the β_{DL} conformer of *N*-formyl-*trans*-2,3-didehydroalaninamide was shown to be the most stable,[761] the geometric and energetic properties of a diamide of serine, HCO-NH-L-$CH(CH_2OH)CO$-NH_2,[762] the conformational preference of acetyl-azaalanine *N*-methylamide,[763] a multivariate calibration method has been reported to determine the chemical composition of binary and ternary mixtures of amino acids based on an Imbrie's Q-mode factor analysis.[764]

6 Chemical Studies of Amino Acids

6.1 Racemisation. – Conditions for enzymic racemisation of D-aspartic acid using on-line coupling of a solid phase extraction column and a ligand-exchange HPLC,[765] and for the production of D-glutamate from L-glutamate using glutamate racemase and L-glutamate oxidase have been described.[766]

The use of racemisation of *N*-(9-(9-phenylfluorenyl))serine-derived cyclic sulfamidates in the synthesis of γ-keto α-amino carboxylates and prolines has also been described.[767]

Mild racemisation conditions using metal complexes have also been reported; the rhodium-catalysed racemisation of *N*-acyl α-amino acids has been reported. The technique will be useful for kinetic resolution processes,[768] and an improved procedure for the racemisation of *N*-acyl α-amino acids uses $Pd(PPh_3)_4$ either as the pre-formed complex or by its formation *in situ*.[769]

6.2 General Reactions of Amino Acids. – *6.2.1 Thermal Stability of Amino Acids.* The stability of selected amino acids under attempted redox constrained hydrothermal conditions has been investigated.[770] The pyrolysis of amino acids has been studied and the recovery of starting materials and the yields of condensation products have been determined; the study aims to shed light on the problem of thermal stability of small biomolecules during their extraterrestrial delivery.[771] The mechanisms of thermal decompositions have attracted interest; the thermo-decomposition of asparamide has been studied.[772] It has been demonstrated that the thermal decomposition of the non-natural amino acid *N*-(tert-butoxycarbonyl)-*p*-fluoro-phenylalanine is slightly different from that of its iodo-analogue in that the dehydration reaction is intramolecular.[773]

6.2.2 Reactions at the Amino Group. Studies on the use of the Fmoc-protecting group continue; a range of N^{α}-protected amino acids have been synthesised using Fmoc as an acylating agent under neutral conditions. The procedure circumvents the oligomerisation that occurs under Schotten-Bauman conditions.[774] 9-Fluorenylmethyl fluoroformate is suggested as a useful reagent for the synthesis of Fmoc amino acids; the products are largely dipeptide free.[775] Fmoc-Serine amide has been prepared by a Schotten-Baumann acylation method from Fmoc-Cl and H.Ser-NH_2.HCl.[776] A simple method for the removal of the Fmoc group has been reported. The method uses catalytic BDU in the presence of aliphatic or polymer-supported thiol.[777]

2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (Nsc) is proposed as a new N-protecting group[778] and the relative merits of the Nsc and Fmoc *N*-protecting groups have been compared.[779] Protection of 3,4-dihydroxyphenylalanine using cyclic ethyl chloroformate is proposed for the hydroxy groups for Fmoc solid phase peptide synthesis.[780] Z- and Boc-Protected amino acids have been prepared using *p*-toluenesulfonyl chloride.[781]

Deprotection of *N*-tert-butoxycarbonyl groups in the presence of tert-butyl esters has been achieved using concentrated H_2SO_4 in t-BuOAc or $MeSO_3H$ in t-BuOAc:CH_2Cl_2. Yields ranged from 70–100%.[782]

The synthesis of orthogonally protected lysine derivatives is reported from lysine and protecting agents DdeOH, ZCl and Alloc-Cl.[783]

Monobenzylation of amino acids occurs at ambient temperature using benzyl chloride in water containing potassium carbonate,[784] while mono-alkylation of *N*-(nitrophenyl sulfonyl) α-amino acid esters under solid-liquid PTC conditions occurred with excellent yields without detectable racemisation.[785] Reaction of L-serine and L-threonine with 2-chloroethanol in aqueous KOH gave *N*,*N*-bis(2-hydroxyethyl)-L-serine and L-threonine.[786]

Maillard reaction compounds have been produced by interaction of amino acids and secondary amines with carbonyls.[787]

Various studies on Schiff base complexes of amino acids have been reported; complexes of cobalt(II), nickel(II), copper(II) and zinc(II) with 2-pyridinecarboxaldehyde and a potentially tridentate amino acid,[788] and some bidentate amino acids are reported,[789] complexes of bidentate Schiff base from *p*-hydroxybenzaldehyde and L-(+)-cysteine;[790] dimethyltin dichloride with amino acid Schiff bases gave 1:2 coordination compounds.[791] The synthesis, mechanism of formation and NMR spectra of lanthanide complexes with an unsymmetrical Schiff base are reported.[792]

Kinetic studies of the interaction of amino acids with aldehydes have also appeared; with vanillin the reactions showed 1st order kinetics.[793] The kinetics of the condensation of glutaraldehyde with amino acids have been studied using UV, pH measurements, microcalorimetry and analysis of functional groups.[794] Reaction of amino acids with *o*-phthalic aldehyde in the presence of sulfite and cyanide ions enabled their determination by spectrophotometric and fluorometric methods.[795]

Studies were reported on the complexes of organo tin(IV) compounds with Schiff bases formed from heterocyclic ketones and amino acids. The resulting compounds were studied by NMR and screened for antibacterial activity.[796]

Various studies of the alkaline permanganate degradation of amino acids have been reported.[797–799]

6.2.3 Reactions at the Carboxy Group. The (2-phenyl-2-trimethylsilyl)ethyl group is proposed as a new carboxy protecting group; the group can be cleaved with tetra-n-butyl ammonium fluoride.[800] The deprotection of t-butyl esters using HNO_3 in CH_2Cl_2 has been investigated. Some unwanted transformations were observed.[801] The electrochemical deprotection of amino acids from their Dim esters is reported.[802]

Boc and Z-Protected amino acid fluorides have been synthesised using DAST as a fluorinating agent.[803]

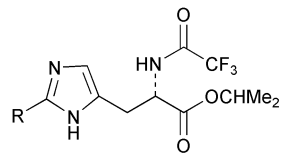
Studies on the decarboxylation of amino acids have been numerous, reported for the reactions themselves, kinetic and mechanistic studies have been reported for the decarboxylation of amino acids by chloramine T,[804] with and without the presence of micelles,[805] and on the role of chlorine in the reaction[806] or for synthetic purposes. A mild and efficient method is reported for the synthesis of 2-substituted pyrrolidinones from amino acids by a tandem radical decarboxylation–oxidation. The reaction proceeds with high yields and good stereoselectiv-

ity.[807] A new synthetic method for the preparation of imides through an oxidative photodecarboxylation reaction of *N*-protected α-amino acids using FSM-16, a mesoporous silica.[808] The anodic oxidation of *N*-acetylisoleucine resulted in a decarboxylation/methoxylation product.[809]

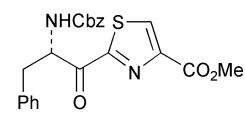
The rate of spontaneous decarboxylation of amino acids has also been studied.[810]

An efficient procedure has been reported for the reduction of α-amino acids to enantiomerically pure α-methyl amines using $LiBH_4$/TMSCl reagent.[811]

A range of imidazoles, including the histidine (**47**), and thiazole (**48**) with chiral side chains derived from amino acids have been prepared from *N*-Cbz-protected α-amino glyoxals. The α-amino glyoxals were obtained from L-amino acids *via* diazo ketones.[812–814]



(47)



(48)

Kinetic studies on basic procedures have been reported on the esterification of L-phenylalanine by methanol,[815] and on the base-catalysed hydrolysis of amino acid esters in the presence of Cu(II)-complexes with a polymer of glutamic acid and ethane-1,2-diol. The rate is enhanced by the presence of these polymers.[816]

6.2.4 Reactions at Both Amino and Carboxy Groups. Kinetic and mechanistic studies have been reported of the oxidative deamination and decarboxylation of L-valine by alkaline permanganate,[817] of silver(I) ion-catalysed oxidative deamination and decarboxylation of D,L-valine by acidic permanganate,[818] of L-amino acids by potassium permanganate in moderately concentrated sulfuric acid; the latter reaction occurs in a two-stage process, both stages first order[819] and of six amino acids by chloramine T.[820]

A study of isotope fractionation during radiation-induced decarboxylation and deamination of L-leucine showed that was more pronounced for $^{13}C/^{12}C$ than for $^{15}N/^{14}N$.[821]

A facile method for the transformation of *N*-(tert-butoxycarbonyl) α-amino acids to *N*-unprotected α-amino methyl esters is reported.[822]

The Dakin–West reaction of *N*-alkoxycarbonyl-*N*-alkyl-α-amino acids employing trifluoroacetic anhydride is reported.[823]

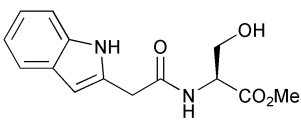
6.2.5 Reactions at the α-Carbon Atom of α- and β-Amino Acids. Other papers under this heading may also appear in the synthesis Sections 4 or in Specific Reactions (6.3), depending on the emphasis of the paper.

The direct asymmetric α-methylation of α-amino acids in two steps has been reported. *N*-protected amino acids were treated with KHMDS followed by MeI in THF/toluene to give high yields with good enantiomeric excess.[824] The stereoselective alkylation of aldimines; prepared from α-amino esters and

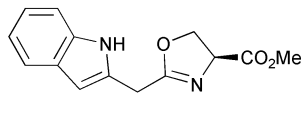
pyridoxal models having an ionophoric side chain composed of a chiral glycerol structure; in the presence of Li^+ or Na^+ gave α,α-dialkyl amino esters after acidic hydrolysis.[825]

The treatment of *N*-MOM-*N*-Boc-α-amino acid derivatives with potassium hexamethyldisilazide followed by methyl iodide under low temperature conditions good yields of the corresponding α-methylated products.[826] Reaction of trifluoracetic anhydride with α-hydroxy acids or α-amino acids in the presence of pyridine was a convenient synthesis of α-trifluoromethylated acyloins.[827]

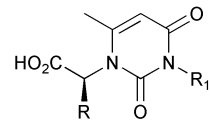
6.3 Specific Reactions of Amino Acids. – For this year's review, in order to obviate the ever swelling size of Section 6.3, which has become something of a 'catch all' section, an attempt has been made to find more specific locations, either in 'Synthesis' or a specific reaction site (*e.g.* Section 6.2) for more of the papers.



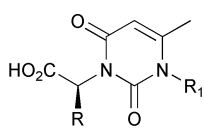
(49)



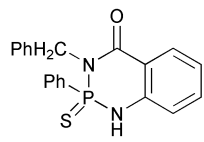
(50)



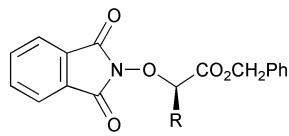
(51)



(52)



(53)



(54)

Reviews of biodegradability characteristics and applications of asparagine acid-[828] glutamic acid-[829] and methylglycine-based chelating agents have appeared.[830] The use of amino acids in the synthesis of heterocyclic compounds continues to prosper. A facile synthesis for heterocycles containing a glycine residue has been reported.[831] A mild and efficient conversion of β-hydroxy amides (**49**) to oxazolines (**50**) is described using DAST and $(MeOCH_2CH_2)NSF_3$ reagents. DAST gives higher yields for serine-containing substrates, whereas $(MeOCH_2CH_2)NSF_3$ gives higher yields for threonine.[832] Z, BOC, FMOC and ALLOC derivatives of 5-aminooxazoles were prepared in one step from acyl amino acids and chlorosulfonyl carbamates.[833] A solid phase procedure, giving a high yield and optical purity, for the synthesis of the uracils (**51**) and (**52**) has been reported, using resin-bound amino acids with isocyanates.[834] A one pot synthesis of the novel 5,11-dioxo-6-methyl-5,9,10,11-tetrahydro-8*H*-naphtho[2,3:1,2]pyrrolizine and its 9-acetoxy analogue[835] and a facile and convenient synthetic method for fluorine-containing 1*H*-pyrrolo[3,2-*h*]quinolines have been reported.[836]

Other novel cyclisation reactions have included an intramolecular defluorinative cyclisation synthesis of difluoromethylated quinazolic acid derivatives,[837] heterocycles of type (**53**) have been produced by the rearrangement, in alcohol, of ester or nitrile derivatives of β-amino acids with the formation of a

β-peptide link.[838] The reaction of aspartic acid derivatives with Grignard reagents yielded γ,γ-disubstituted α- and β-aminobutyrolactones.[839] Optically active β-amino acid *N*-carboxyanhydrides have been synthesised through cyclising N^{β}-Boc β-amino acids using PBr_3.[840] The D-α-(phthaloylamino)oxy acids (**54**, R = iso-Pr, sec-Bu, CH_2Ph, CH_2CONH_2) were synthesised using a Mitsunobo reaction from L-amino acids with inversion of configuration.[841] Papers have reported the conversion of amino acid derivatives to alkaloids or their precursors; *via* *N*-acyliminium ions generated in a one-pot radical decarboxylation–oxidation[842] and heterocyclic-β-amino esters were shown to be diastereoselectively alkylated with alkyl halides to lead to direct precursors of bicyclic alkaloids.[843]

Studies of the reactions of amino acids with other natural products, heterocycles and other compound types have continued. The synthesis of amino acid derivatives of 7-methoxycarbonylneoflavones,[844] optically active derivatives methylated 5-amino-azaheterocycles[845] and naphthalene-1,2-dione-amino acid adducts have been reported.[846] Amino acid-estradiol derivatives have been synthesised enzymatically for the first time using a protease-catalysed condensation.[847]

Complexes of adducts of amino acids with nucleobases and of their model compounds have been discussed.[848] The formation of a *N*-glycosidic linkage between *N*-acetylglucosamine and asparagines, using aspartic acid γ-fluoride in combination with either glycosyl azide or Bu_4NF, has been investigated.[849] Diaryl-selenides and selenones containing amino acid moieties have been synthesised from 4′-nitro-4-aminodiphenylselenide.[850]

The kinetics and mechanism of the reaction between amino acids and stable free radicals derived from 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) have been investigated. The reaction has a three-step mechanism with complex kinetics.[851]

Amino acids are used as ligands or supports in various reactions. The synthesis of polymer supported α-amino acids and their application in the alkylation of arenes has been described.[852] Enantioselective Si–H insertion of methyl phenyldiazoacetate catalysed by dirhodium(II) carboxylates incorporating *N*-phthaloyl-(*S*)-amino acids as chiral bridging ligands has been reported.[853]

Some phosphorus-containing derivatives and analogues of amino acids do not fit snugly into the section on synthesis of compounds with phosphorus in the side chain. They are reported here. 5′-*O*-Derivatisation of AZT with the *O*-methyl esters of phenylalanine and tryptophan gave 5′-amino acid phosphoramidothioates.[854]

Sulfamates, R-X-SO_2-NH_2 (X = O, NH), derived from amino acids, have been shown to react with trialkyl phosphates, in the presence of diisopropylazodicarboxylate, to give phospha-λ^5-azenes which undergo an imidate–amidate rearrangement to yield *N*-phosphorylsulfamates, bioisosteres of pyrophosphate.[855] The synthesis has been reported of *N*-alkyl-(α-aminoalkyl)phosphine oxides and phosphonic esters, *e.g.* $(MeO)_2P(O)CHPhNHCH(CH_2CHMe_2)CO_2CH_2Ph$ starting from α-amino acids.[856] Condensation of allylated amino acids with methyl or vinyl phosphonic dichlorides gave rise to three diastereomeric P-chiral amino

acid-derived phosphonamidic anhydrides. The mechanism of the reaction is discussed.[857] 2-Hydroxy esters of oxophosphorus acid react with glycine to give amidoglycine H-phosphinate and cyclic phosphoamido anhydrides.[858]

The kinetics and products of the thiophosphorylation of histidine have been reported.[859] The Michael addition reactions of *O*-quinone methide, generated thermally and photochemically in water, to amino acids and glutathione to give alkylated products has been reported[860] and that between chiral Ni(II) complex of glycine and 3-(*trans*-enoyl)oxazolidin-2-ones have been reported. The latter show electron donor–acceptor attractive interaction-controlled face diastereoselectivity.[861] Similar addition reactions involving allyl groups, *e.g.* diastereoselective addition of allyl reagents to variously *N*-protected L-alanals,[862] and the reaction of allyl isothiocyanates with amino acids and peptides in model aqueous systems have been studied. The latter reactions are pH-dependent. Mechanisms of the observed reactions are proposed.[863]

Complexes/compounds of amino acids with metals can be divided into two types, those containing an amino acid and the metal and those containing a third component (tertiary complexes).

The coordination chemistry of amino acids with platinum and palladium has been reviewed[864] and the preparation of chiral cyclopalladated liquid crystals from amino acids has been described.[865]

Studies of binary complexes have included the formation of complexes between L-carnosine and Cu(II) and their role as catalysts in the hydrolysis of amino acid esters,[866] synthesis and characterisation of manganese(II), cobalt(II), nickel(II) and palladium(II) complexes of D,L-aspartic acid,[867] a method for attaching organometallics to the C-terminus of amino acids *via* a Pd-catalysed, two step procedure is presented,[868] the preparation and reactions of stannylated amino acids,[869] stereoselective synthesis of ferrocenyl amino acids,[870] synthesis and characterisation of La(III) solid complex with L-hydroxyproline,[871] and gold complexes with glycine, histidine and tryptophan. The antimicrobial activity of the complexes is reported.[872]

The reaction of lysine with 18-molybdophosphate to give a salt formulated as $(Lys)_2H_6[P_2Mo_{18}O_{62}]16H_2O$ has been reported.[873]

The complexation of asparagine by dioxovanadium(V) has been studied and the stability constants measured.[874]

A thermochemical study has been reported of the solid phase coordination reaction of glycine and copper hydroxide.[875] The kinetics and mechanism have been studied of the reactions of bis(guanide)copper(II) with amino acids in aqueous media.[876]

Tertiary complexes of Cu(II) and Zn(II) with 2,2′-bipyridal as a primary ligand and amino acids as secondary ligands are reported.[877] Amino acid-derived organozinc reagents have been coupled with aryl triflates at room temperature using palladium catalysts.[878] The complexation of individual amino acids, and amino acids in general, with various metals has been studied over the time period; specifically, complexation of praseodimium and calcium cations with *N*-benzoyl glutamic acid.[879]

The reaction of glycinatocopper complexes with cinnamaldehydes under

mildly basic conditions gave polysubstituted prolines which can be systematically modified in a number of chemoselective transformations[880] and new chiral ligands derived from (*S*)-leucine for the enantioselective addition of diethyl zinc to aldehydes have been described.[881] The reactivity of peroxo α-amino acid (glycine, alanine, valine and leucine) complexes of molybdenum(VI) towards nitric oxide and carbon dioxide in water solutions has been investigated.[882] The structure of a metallated NCA product and its role in polypeptide synthesis involved in the reactions of α-amino acid-*N*-carboxyanhydrides (NCAs) with organometallic palladium(0) and platinum(0) compounds has been investigated.[883]

The decomposition of an amino acid cupric complex using tetrahydrothiazole-2-thione for the preparation of N^{α}-Boc-N^{ε}-Fmoc-L-lysine is reported.[884]

There have been many papers on the subject of oxidation, most of them fairly routine. Summarising, kinetics and mechanism of the oxidation of various amino acids have been reported; L-cysteine, L-cystine and *N*-methyl-L-cysteine by potassium ferrate;[885] D-cycloserine by sodium *B*-bromo-*p*-toluenesulfonamide in acid,[886] L-(+)-aspartic acid by diperiodatonickelate(IV) in aqueous alkaline medium[887] and acidic Mn(III) has been used to oxidise phenylalanine,[888] and L-lysine.[889] A series of papers have appeared on the oxidation of various amino acids by vanadium(V) in a micellar system in the presence of sulfuric acid.[890–894] The kinetics and mechanism of oxidation of α-amino acids by benzyltrimethylammonium chlorobromate,[895] and of methionine by hexamethyltetramine-bromine[896] have been studied. Methionine has also been oxidised by peroxynitrite.[897]

Kinetic studies of the oxidation of cysteine in oxygen-saturated aqueous solution in the presence of Cu(II)-containing polymers,[898] and its autoxidation catalysed by copper complexes have been reported. The latter study indicated that catecholamines stimulated the process.[899]

The electroreflectance (ER) technique has been applied to the study of the electrooxidation of some simple amino acids on a Pt(III) surface in acid medium[900] and an electrocatalytic oxidation reaction at a copper microelectrode has been described, which allows the detection of underivatised peptides and amino acids using sinusoidal voltammetry.[901] The oxidation of protein amino acids by free stable hydrazyl radicals (*e.g.* 2-*p*-phenylsulfonic acid 2-phenyl picrylhydrazyl Na salt) has been studied spectrophotometrically.[902]

The ozone oxidation products of amino acids and small peptides have been identified by Electrospray mass and Tandem mass spectrometry.[903] Diastereoselective sulfoxidation of methionine and cysteine derivatives in supercritical CO_2 shows a dramatic pressure dependence; the major product was found to be 'anti'.[904]

Reduction of amino acids has also been widely reported. A simple method for the reduction of carboxylic acids to aldehydes or alcohols using H_2 and Pd/C[905] and the reduction of amino acids containing a hydroxy side chain to β-amino alcohol and the preparation of their peptide alcohols have been studied.[906] Enantiomerically pure 2-amino alcohols have also been prepared by the reduction of α′-(*N*-Boc)amino β-ketosulfoxides,[907] while *syn*-γ-hydroxy-α-amino acids

have been derived from stereoselective sodium borohydride reduction of γ-oxo-α-amino acids catalysed by manganese(II) chloride.[908] A study has shown that the one-electron reduction of selenomethionine oxide occurs more readily than for its sulfur analogue, methionine oxide.[909]

Asymmetric hydrogenation of unsaturated amino acids has been performed using a new aminophosphine phosphinite ligand derived from ketopinic acid as a catalyst,[910] and rhodium complexes with chiral 4-(diphenylphosphanyl)-1-(dialkylamino)butane in the presence of SDS have been investigated. Stereoselectivity was found to be higher in water than in methanol.[911] The mechanism for the homogeneous hydrogenation of dehydroamino acids has been deduced using information based on kinetic studies and NMR characterisation.[912]

A number of reactions do not fit comfortably into other categories and so they are listed here. An improved method for cysteine alkylation, involving the refluxing the cysteine thiol with the appropriate alkyl bromide in a solution of sodium ethoxide in ethanol, is reported.[913]

Isomeric 4-prolinyl and 4,4-diprolinyl amines have been synthesised from 4-epimeric *N*-Boc-4-hydroxyproline tert-butyl esters.[914] The synthesis of *N*-α-amino aldehydes from their morpholine amide derivatives is reported.[915]

A Mannich-type reaction of imines with *N*-protected amino acid chlorides has been found to give good stereoselectivity (99%) using *N*,*N*-phthaloyl-tert-leucine as a chiral auxiliary.[916]

Various reactions, including β-fragmentation reactions, involving aminyl radicals from amino acids have been reported.[917] The synthesis of aspartic acid derivatives useful for the preparation of misacylated transfer RNAs has been described.[918]

The kinetics have been reported of the reaction of sodium glycinate with benzoyl chloride under inverse phase transfer catalysis.[919]

6.4 Effects of Electromagnetic Radiation on Amino Acids. – Irradiation studies have concentrated this year on alanine; two studies using EPR of irradiated alanine,[920] one concentrating on relaxation rates of stable paramagnetic centres.[921] *Ab initio* and semi-empirical methods were used to model radical formation in L-alanine after irradiation; mechanisms of radical formation were deduced.[922]

An efficient nucleophilic substitution reaction of aryl halides with amino acids under focused microwave radiation has been reported.[923]

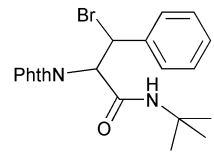
Laser flash photolysis has been use to study a number of reactions; 4-nitroquinoline-1-oxide with D-methionine and its dipeptides have been studied using 248 nm laser flash photolysis,[924] the mechanism of the pyrene sensitised photodecomposition of *N*-phenylglycine depends on the addition of an acceptor as additive.[925] The pH dependence of the photoionisation of aromatic amino acids[926] and the characterisation of transient species of aromatic amino acids using acetone as photosensitiser under laser photolysis have been reported.[927]

A nanosecond laser flash photolysis study is reported of the fast decarboxylation of aliphatic amino acids induced by 4-carboxybenzophenone triplets in aqueous solution. The transfer of protons from aminium radicals within the

solvent cage gives rise to aminyl radicals, which undergo β-decarboxylation. The rate constant for this reaction is an order of magnitude above that observed for the decarboxylation of acyloxy radicals in aqueous media.[928] Supersaturated aqueous solutions of glycine exposed to intense pulses of plane-polarised laser light have been shown to crystallise unexpectedly into the γ-polymorph of glycine.[929]

UV photolysis of protected glycines in the presence of di-tert-butyl peroxide, benzophenone and substituted toluenes lead to selective alkylation at the α-position.[930] The synthesis and characterisation of a photolabile precursor of glycine is reported. The photolysis of the caged-glycine is reported and is proposed as a useful tool for the investigation of the glycine receptor.[931]

The photoionisation characteristics have been reported of amino acids covalently tethered to a naphthol chromophore. The chromophore was separated from the amino acid by an alkyl chain.[932] The photo-induced electron-transfer of ruthenium complexes with derivatised proline residues has been studied.[933] The mechanism of the photolysis reaction of *N*-bromo-*N*-tert-butyl-N^{α}-phthaloylphenylalaninamide to give a 1:1 mixture of the diastereoisomers of 3-bromo-*N*-tert-butyl-N^{α}-phthaloylphenylalaninamide (**55**) is reported.[934]



(55)

The mechanism of pH-dependent photolysis of aliphatic amino acids and enantiomeric enrichment of racemic leucine by circularly polarised light is investigated.[935] An enantioselective fluorescence effect that can be used for determining the optical purity of proline has been reported. The method uses copper(II) complexes of modified cyclodextrins.[936] Fluorescence-quenched ternary complex Cu^{2+}/4-(dimethylamino)benzonitrile/β-cyclodextrin interacted with glutamate to restore the fluorescence.[937] High pressure was found to shift the fluorescence spectra of tryptophan and its derivatives to the red direction, mechanisms for the shift were discussed.[938]

7 Analytical Methods

7.1 Introduction. – Reviews have appeared of the column chromatography,[939] and mass spectrometry and GCMS of phosphorus analogues of amino acids.[940]

7.2 Gas–Liquid Chromatography. – The optimum conditions have been reported for the analysis of amino acid esters by GC using a flame ionisation detector.[941] GC–MS methods for the analysis of stable isotope-labelled amino acids in biological samples continue to attract interests. Methods for the deter-

mination of stable isotope-labelled cysteine and glutathione in biological samples[942] and for the simultaneous determination of isotopic enrichments of ^{13}C labelled homocysteine and methionine in human plasma by GC-negative chemical ionisation MS has been reported.[943] A procedure has been reported for the spectrophotometric determination of aromatic and heterocyclic amino acids in mixtures using the Vierordt method.[944] A GC–MS study has been reported of trimethylsilyl/t-butyldimethylsilyl derivatives of amino acids in model systems.[945]

7.3 Ion-exchange Chromatography. – Amino acid analysis (especially using ion exchange column chromatography) and its relevance to the silk industry has been reviewed.[946]

Amperometric determination has been shown to be useful for the determination of underivatised amino acids at a nickel-modified gold electrode by anion exchange chromatography.[947]

Various theoretical studies of ion exchange chromatography have been reported. Various calculations and theoretical models have been performed on the molecular sorption of amino acids on ion-exchange resins. The calculations were suitable for the prognostication of the selectivity of the ion-exchange sorption of amino acids.[948–950] Electric mass transfer of amino acids through ion exchange membranes has been modelled experimentally by the laser interferometry method. Direct proof has been obtained for the barrier effect in electrodialysis of amino acids.[951]

Ion exchange equilibria of amino acids on strong anionic resins in the hydroxide form have been reported.[952] A study has been reported of the separation of amino acids by displacement chromatography using carbon dioxide as a displacer.[953] A report has appeared of the desalination of a mixture of amino acids using salt-type polystyrene-based strongly acidic cation exchange resin using H_2O as eluant so that the resin did not need regenerating.[954]

7.4 Thin-layer Chromatography. – The thin-layer chromatographic behaviour of twenty-four amino acids was examined on plain silica gel and impregnated with cationic and anionic solutions using water-in-oil microemulsions as mobile phase.[955] A new chiral β-cyclodextrin-bonded stationary phase substituted by 3,5-dinitrobenzoyl groups has been reported for the separation of dansyl amino acid enantiomers.[956]

The TLC properties of sulfur-containing amino acids have been compared with their phosphonic analogues.[957] A reversed phase TLC study has been reported of the interaction of fourteen hydrazines with amino acids and Bovine serum albumin.[958]

7.5 High Performance Liquid Chromatography. – The separation and determination of amino acids in food by HPLC have been reviewed. [959]

The application of LC/MS to the determination of absolute configuration of amino acids has been reviewed.[960]

An HPLC separation system for underivatised amino acids has been coupled

with a fluorescence detection system, giving detection limits of the order of 10 ppm[961] and a system of indirect amperometric detection for these materials for use in microcolumn liquid chromatography has been described.[962]

A procedure has been reported for the determination of amino acids in human blood serum using reversed phase HPLC[963] and HPLC-UV has been used to determine the amount of lysine in a lysine hydrochloride injection.[964]

Chiral HPLC has been used for enantiomeric separation of α-methyl-α-amino acids. Two different methods were employed for derivatised and underivatised compounds.[965] HPLC methodologies using a chiral stationary phase based on the glycopeptide antibiotic teichoplanin has been used for the separation of stereoisomeric cyclic β-substituted α-quaternary α-amino acids,[966] for enantioseparation of *N*-(tert-butyloxycarbonyl)amino acids,[967] for the recognition of amino acids and structurally related compounds,[968] and a procedure for the determination of the chiral purity of synthetic amino acids by HPLC has also been described.[969]

When phenylthiohydantoin derivatives of α-amino acids were separated using polysaccharide-based chiral stationary phases, it was reported that Chiralcel OF preferentially retained D-isomers, whereas Chiralpak AS was better for L-isomers.[970] Enantiomeric and diastereomeric HPLC separation of cyclic β-substituted α-quaternary α-amino acids (cycloalkanecarboxylic acids) was achieved on a copper(II)-D-penicillamine chiral stationary phase. Optimum conditions for the separation of the four possible stereoisomers of each compound in a single run were investigated[971] and the direct HPLC enantioseparation of *N*-protected β-methyl-substituted unusual amino acids on a quinine-derived chiral anion-exchange stationary phase has been described and the effects of different protecting groups were investigated.[972] Various methods of derivatisation have been reported. In order to analyse the various amino acids in rumen fluid, samples were derivatised with 9-fluorenylmethyl chloroformate and separated with a methanol gradient in sodium citrate buffer,[973] *N*-hydroxysucciminidyl-α-naphthyl acetate has been proposed as a precolumn derivatisation reagent for the separation and determination of amino acids by reverse phase HPLC[974] and pre-column derivatisation of amino acids by *o*-phthaldialdehyde/mercaptoethanol and Fmoc with two fluorescence detectors followed by HPLC separation[975]

Methods have been reported for the simultaneous determination of L-phenylalanine and branched chain amino acids in plasma by LC with a co-immobilised enzyme reactor and fluorescence detection[976] and the temperature-responsive chromatographic separation of amino acid phenythiohydantoins using aqueous media as the mobile phase HPLC using modified silica gel with functional polymers. The polymer-grafted surface exhibits temperature regulated hydrophobic/hydrophilic properties changes in water.[977]

The effect of the size of the alkyl substituent on the ester group of benzoyl derivatives of amino acids on the selectivity of the stationary phase (*R*)-3,5-dinitrobenzoylphenyl glycine and binary nonaqueous eluents has been investigated.[978]

Synthetic β-heterocyclic and β-naphthyl alanines and phenylalanines have

been separated on reversed-phase HPLC after derivatisation with FDNP-Val-NH_2. The L-isomers were eluted faster, providing a determination of chiral purity.[979]

7.6 Capillary Zone Electrophoresis (CZE) and Related Analytical Methods. – There is some overlap in this section with the separation of enantiomers of amino acids. Generally, if the emphasis is on the technique, then the paper appears here.

Amendments to the techniques of capiliary electrophoresis have included; sample pre-concentration by filed amplification stacking for microchip-based capillary electrophoresis has shown up to 20-fold signal gains,[980] pressurised gradient capillary electrochromatography for the separation of eighteen amino acids,[981] the development of glass microchips, integrating chemical derivatisations, electrophoretic separations and end column amperometric detections for measurements of amino acids.[982] Amino acids have been separated using planar capillary electrochromatography with an integrated fritless column and conventional stationary phase.[983]

Amino acids have been separated by aqueous two-phase electrophoresis coupled to traditional extraction[984] and using two-phase electrophoresis with dextran-polyethylene glycol-water as a working system.[985] Amino acid enantiomers have also been separated using two-dimensional capilliary electrophoresis coupled to TLC. The TLC plates, which used a mobile phase containing a high concentration of β-cylodextrins, were imaged by laser-induced fluorescence.[986]

A new detection technique exploiting indirect fluorescence has been adapted to the electrophoretic microchip to provide fast analysis of amino acids; sensitivity was lower than previous methods, but the ease of use makes the system attractive.[987] Amino acids and peptides have also been fluorescently labelled, concentrated in organic solvent, separated by CZE and detected by fluorescence,[988] while fluorescein isothiocyanate-labelled amino acids have been separated by capillary electrophoresis with laser-induced fluorescence detection.[989]

N-Dansyl amino acids have been detected on capillary electrophoresis–chemiluminescence analysis using peroxyoxalate reagent. The method had a detection limit of 1×10^{-8} M for *N*-dansyltryptophan.[990]

An on-column derivatisation and analysis of amino acids, peptides and alkylamines by anhydrides has been performed using capillary electrophoresis.[991] An analysis of amino acids from peptide and protein hydrolysates after derivatisation with phenylisothiocyanate is reported using capillary electrophoresis using SDS in phosphate buffer. The results show a 20-fold increase in sensitivity over the HPLC method.[992]

Developments have been reported in the experimental procedures for the chiral separation of amino acid derivatives using capillary electrophoresis. The study indicated that the best experimental conditions varied for each compound analysed.[993] Investigations of the separation of chiral acids[994] and *N*-derivatised amino acids[995] using enantioselective non-aqueous capillary electrochromatography systems have been reported. Chiral separation of amino acids has been achieved by ligand exchange capillary electrochromatography using continuous

beds.[996] Various chiral selectors have been applied to the separation of enantiomers by capillary electrophoresis. Amphiphilic aminosaccharide derivatives have been used as chiral selectors in capillary electrophoresis; their selectivity differed for dansyl amino acids[997] and teichoplanin has been applied to a background electrolyte for enantioseparation of *N*-(tert-butyloxycarbonyl)amino acids as well as to the stationary phase on HPLC (see also Section 7.5).[998] Enantioseparation of anionic analytes by non-aqueous capillary electrophoresis using quinine and quinine derivatives as chiral counter ions for *N*-protected amino acids using benzoyl, nitrobenzoyl and nitrobenzyloxycarbonyl protecting groups has been reported.[999]

Overlapping peaks of amino acid derivatives in capillary electrophoresis have been resolved using multivariant curve resolution based on alternating least squares.[1000] Phenylalanine, isoleucine and tyrosine derivatives of 1,2-naphthoquinone-4-sulfonate were found to be only partially separated by capillary electrophoresis. Partial least squares regression overcomes lack of selectivity for these amino acids. Histidine and leucine derivatives were not separated.[1001]

7.7 Assays for Specific Amino Acids. – The methods of determination of total homocysteine in plasma have been reviewed.[1002] Automatic immunoassay for total plasma homocysteine using a competitive fluorescent polarisation technique has been reported.[1003] L-Cysteic acid has been analysed by reversed-phase HPLC.[1004] An assay is reported for 3-nitrotyrosine in biological tissues and fluids using combined liquid chromatography and tandem mass spectrometry. It is reported that under appropriate conditions 3-nitrotyrosine is formed as an artefact in sample extraction and derivatisation.[1005]

8 References

1. 'Amino Acid Derivatives', G. C. Barrett (ed.), OUP, 1999.
2. 'Comprehensive Natural Product Chemistry, Vol 4: Amino Acids, Peptides, Porphyrins and Alkaloids', J. W. Kelly (ed.), Elsevier, 1999.
3. 'Aminophosphonic and Aminophosphinic Acids: Chemistry and Biological Activity', V. P. Kukhar and H. R. Hudson (eds.), Wiley, 2000.
4. P. Righi, *Semin. Org. Synth.*, Summer School, 'A Corbella', 24th, 1999, 401.
5. D. J. Ager, I. G. Fotheringham and N. J. Turner, *Spec. Chem.*, **20**, 2000, 46.
6. M. Zamfir, *Rev. Chim* (*Bucharest*), **50**, 1999, 826; *Chem. Abs.*, **133**, 17754.
7. R. Broxterman, T. Sonke, H. Wories and W. Van den Tweel, *Pharm. Manuf. Int.*, 2000, 61.
8. I. Fotheringham, *Biotechnology*, **8b**, 2000, 313.
9. J. Ogawa and S. Shimizu, *Stereosel. Biocatal.*, 2000, 1.
10. I. Fotheringham, *Curr, Opin. Chem. Biol.*, **4**, 2000, 120.
11. R. M. Williams, *Methods Mol. Med.*, 1999, 23.
12. O. Kobayashi and H. Ishitani, *Gendai Kagaku*, **348**, 2000, 34; *Chem. Abs.*, **133**, 286282.
13. Y. Zheng, *Daxue Huaxue*, **14**, 1999, 26; *Chem. Abs.*, **132**, 251380.
14. H.-Q. Zhan, *Guangxi Huagong*, **29**, 2000, 19; *Chem. Abs.*, **134**, 168121.

15. L. A. Wessjohan, *Curr. Opin. Chem. Biol.*, **4**, 2000, 303.
16. A. J. M. Wagenmakers, *Proc. Nutr. Soc.*, **58**, 1999, 987.
17. T. Shioiri and Y. Hamada, *Syn. Lett.*, 2001, 184.
18. N. Sewald, *Bioorg. Chem.*, 1999, 193.
19. A. Senning, *Dan. Kemi*, **81**, 2000, 24; *Chem. Abs.* **134** 147806.
20. H. Li and A. Zhang, *Dizhi Diqiu Huaxue*, **28**, 2000, 76; *Chem. Abs.*, **133** 153283.
21. E. Andersson, B. R. T. Simoneit and N. G. Holm, *Appl. Geochem.*, **15**, 2000, 1169.
22. P. Mastalerz and P. Kafarski, *Aminophosphonic Aminophosphinic Acids*, 2000, 1.
23. V. Plasman, J. C. Braekman, D. Daloze, D. Windsor and J. M. Pasteels, *J. Nat. Prod.*, **63**, 2000, 1261.
24. A. V. Perepelov, S. N. Senchenkova, M. Cedzynski, A. Ziolkowski, E. V. Vinogradov, W. Kaka, A. S. Shashkov and Y. A. Knirel, *Carbohydr. Res.*, **328**, 2000, 441.
25. T. Selmer, J. Kahnt, M. Goubeaud, S. Shima, W. Grabarse, U. Erlmer and R. T. Thauer, *J. Biol. Chem.*, **275**, 2000, 3755.
26. Y.-W. Guo and E. Trivellone, *J. Asian Nat. Prod. Res.*, **2**, 2000, 251.
27. F. Cafieri, E. Fattorusso, O. Taglialatela, M. Di Rosa and A. Ianora, *Tetrahedron*, **55**, 1999, 13831.
28. P. Ciminiello, C. Dell'Aversano, E. Fattorusso, S. Magno and M. Pansini, *J. Nat. Prod.*, **63**, 2000, 263.
29. J. B. MacMillan and T. F. Molinski, *J. Nat. Prod.*, **63**, 2000, 155.
30. S. E. Brantley and T. F. Molinski, *Org. Lett.*, **1**, 1999, 21650.
31. Y. Murata and N. U. Sato, *J. Agric. Food Chem.*, **48**, 2000, 5557.
32. W. A. Gallimore, D. L. Galario, C. Lacy, Y. Zhu and P. J. Scheuer, *J. Nat. Prod.*, **63**, 2000, 1022.
33. A. Qureshi, P. L. Colin and D. J. Faulkner, *Tetrahedron*, **56**, 2000, 3679.
34. S. Liao and V. J. Hruby, *Methods Mol. Med.*, 1999, 175.
35. D. Tourwe, K. Iterbeke, W. M. Kazmierski and G. Toth, *Methods Mol. Med.*, 1999, 321.
36. R. H. Dodd, *Molecules*, **5**, 2000, 293.
37. D. B. Berkowitz, J. M. McFaddon, M. K. Smith and M. L. Pedersen, *Methods Mol. Med.*, 1999, 467.
38. N. A. Sasaki, *Methods Mol. Med.*, 1999, 23.
39. G. Zvilichovsky and V. Gurvich, *Recent Dev. Org. Chem.*, **3**, 1999, 87.
40. D. Ferraris, *Diss. Abstr., Int, B*, **60**, 2000, 5520.
41. R. Wu, C. C. Orji, R. Michakczyk, J. G. Schmidt, Z. Li, E. M. Stocking, D. A. Ashburn, J. A. Kelly, O. Khalsa, R. A. Martinez, S. V. S. Mariappan, C. J. Unkefer and L. A. P. Silks, *Recent Res. Dev. Org. Bioorg. Chem.*, **3**, 1999, 1.
42. R. L. Polt, *Amino Acid Deriv.*, 1999, 101.
43. J. Huang and R. Chen, *Heteroat. Chem.*, **11**, 2000, 480.
44. M. Barbaste, V. Roland-Fulcrand, M.-L. Roumestant, P. Viallefont and J. Martinez, *Pept.* 1998, *Proc. Eur Pept. Symp.*, 25*th*, 1999, 110.
45. T. A. Ayers and T. V. Rajan-Babu, *Process Chem. Pharm. Ind.*, 1999, 173.
46. J. F. Liebman, H. Y. Afeefy and S. W. Slayden, 'Amide Linkage', A. Greenburg (ed.), John Wiley and Sons, NY, 2000, 115.
47. J. Yong, D. Y. Qin and Y. F. Zhao, *Chin. Chem. Lett.*, **10**, 1999, 537.
48. P. Karfarski and J. Zon, *Aminophosphonic Aminophosphinic Acids*, 2000, 33.
49. V. P. Kukhar, *Aminophosphonic Aminophosphinic Acids*, 2000, 75.
50. S. Lall and R. Engel, *Aminophosphonic Aminophosphinic Acids*, 2000, 103.
51. V. P. Kukhar, *Aminophosphonic Aminophosphinic Acids*, 2000, 127.
52. H. Fu and Y. F. Zhao, *Huaxue Xuebao*, **58**, 2000, 6; *Chem. Abs.*, **132**, 166254.

53. G. Mitulovi, M. Lammerhofer, N. M. Maier and W. Lindner, *J. Labelled Compd. Radiopharm.*, **43**, 2000, 449.
54. N. Valls, M. Lopez-Canet, M. Vallribera and J. Bonjoch, *J. Am. Chem. Soc.*, **122**, 2000, 11248.
55. H. Miyabe, M. Ueda, N. Yoshioka, K. Yamakawa and T. Naito, *Tetrahedron*, **56**, 2000, 2413.
56. V. A. Soloshonok, *ACS Symp. Ser.*, 746 (*Asymmetric Fluoro-organic Chemistry*), 2000, 74.
57. A. G. Myers, D. W. Kung, B. Zhong, M. Movassaghi and S. Kwon, *J. Am. Chem. Soc.*, **121**, 1999, 8401.
58. H. Ishitani, S. Komiyama, Y. Hasegawa and S. Kobayashi, *J. Am. Chem. Soc.*, **122**, 2000, 762.
59. M. Turks, D. Katkevica, H. Smits and E. Bizdena, *Collect. Symp. Ser.* 2, 1999, 268.
60. H. Zhang, P. Xai and W. Zhou, *Tetrahedron: Asymmetry*, **11**, 2000, 3439.
61. Z. Zhang, A. Van Aerschot, C. Hendrix, R. Busson, F. David, P. Sandra and P. Herdewijn, *Tetrahedron*, **56**, 2000, 2513.
62. J. T. Lundquist, K. S. Orwig and T. A. Dix, *J.Org. Chem.*, **64**, 1999, 9265.
63. D. J. Ager, I. G. Fotheringham, T. Li, D. P. Pantaleone and R. F. Senkpeil, *Enantiomer*, **5**, 2000, 235.
64. A. J. Davies, M. S. Ashwood and I. F. Cottrell, *Synth. Commun.*, **30**, 2000, 1095.
65. S. Yao, S. Saaby, R. G. Hazell and K. A. Jorgensen, *Chem. Eur. J.*, **6**, 2000, 2435.
66. P. Merino, S. Franco, F. L. Merchan and T. Tejero, *Synlett*, 2000, 442.
67. R. Chinchilla, P. Mazon and C. Najera, *Tetrahedron: Asymmetry*, **11**, 2000, 3277.
68. D. Cahard, B. Thierry and J.-C. Plaquevent, *Proc. ECSOC*-3; *Proc. ECSOC*-4, 1999–2000, 691; *Chem. Abs.*, 2001, 133335.
69. G. Guillena and C. Najera, *J. Org. Chem.*, **65**, 2000, 7310.
70. C. S. Park, H. G. Choi, H. Lee, W. K. Lee and H.-J. Ha, *Tetrahedron: Asymmetry*, **11**, 2000, 3283.
71. G. Burtin, P.-J. Corringer and D. W. Young, *Perkin* 1, 2000, 3451.
72. T. Abellan, R. Chinchilla, N. Galindo, C. Najera and J. M. Sansano, *J. Heterocycl. Chem.*, **37**, 2000, 467.
73. T. Ooi, M. Takeuchi, M. Kameda and K. Maruoka, *J. Am. Chem. Soc.*, **122**, 2000, 5228.
74. G. S. Currie, M. G. B. Drew, L. M. Harwood, D. J. Hughes, R. W. A. Luke and R. J. Vickers, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2982.
75. K. Iterbeke, J. Severeyns, P. Defreyn and D. Tourwe, *Pept.* 1988, *Proc. Eur. Pept. Symp.* 25*th*, 1999, 666.
76. Y. N. Belokon, R. G. Davies and M. North, *Tetrahedron Lett.*, **41**, 2000, 7245.
77. A. Jourdant and J. Zhu, *Tetrahedron Lett.*, **41**, 2000, 7033.
78. K. W. Laue, S. Kroger, E. Wegelius and G. Haufe, *Eur. J. Org. Chem.*, 2000, 3737.
79. M. Moreno-Manas, E. Trepat, R. M. Sebastian and A. Vallribera, *Proc. ECSOC*-3; *Proc. ECSOC*-4, 1999, 41; *Chem. Abs.*, **132**, 133187.
80. G. Jones, D. C. D. Butler and C. J. Richards, *Tetrahedron Lett.*, **41**, 2000, 9351.
81. V. Alezra, M. Bonin, A. Chiaroni, L. Micouin and C. Riche and H.-P. Husson, *Tetrahedron Lett.*, **41**, 2000, 1737.
82. F.-Y. Zhang and E. J. Corey, *Org. Lett.*, **2**, 2000.
83. M. Hasegawa, D. Taniyama and K. Tomioka, *Tetrahedron*, **56**, 2000, 10153.
84. H. Fu and Y. F. Zhao, *Huaxue Xuebao*, **58**, 2000, 6; *Chem. Abs.*, **132**, 166254.
85. S. Lall and R. Engel, *Aminophosphonic Aminophosphinic Acids*, 2000, 103.
86. V. P. Kukhar, *Aminophosphonic Aminophosphinic Acids*, 2000, 127.

87. C. Papa and C. Tomasini, *Eur. J. Org. Chem.*, 2000, 1569.
88. O. Tamura, H. Sugita, N. Mita, Y. Uyama, N. Morita, M. Ishiguo, T. Kawasaki, H. Ishibashi, M. Sakamoto, *Synlett*, 2000, 1553.
89. M. Takamura, Y. Hamashima, H. Usuda, M. Kanai and M. Shibasaki, *Chem. Pharm. Bull.*, **48**, 2000, 1586.
90. P. D. Bailey, G. M. Rosair, D. Taylor and I. M. McDonald, *Chem. Commun.* (*Cambridge*), 2000, 2451.
91. S.-B. Chen, Y.-M. Li, S.-Zhong Luo, G. Zhao, B. Tan and Y.-F. Zhao, *Phosphorus, Sulfur Silicon Relat. Elem.*, **164**, 2000, 277.
92. J.-F. Tang, J.-Y. Zhou, Z. I. Liu, Y.-X. Xiao, Z.-H. Wu and K.-N. Wu, *Wuhan Daxue Xuebao*, *Ziran Kexueban*, **45**, 1999, 861; *Chem. Abs.*, **132**, 264248.
93. A. P. A. Arbore, D. J. Cane-Honeysett, I. Coldham and M. L. Middleton, *Synlett*, 2000, 236.
94. H. Mues and U. Kazmaier, *Proc ECSOC*-3; *Proc. ECSOC*-4, 1999–2000, 1324; *Chem. Abs.* **132**,138697.
95. I. Savage, E. J. Thomas and P. D. Wilson, *J. Chem. Soc., Perkin Trans. 2*, 1999, 3253.
96. U. Kazmaier, *J. Indian Chem Soc.*, **76**, 1999, 631.
97. Y.-S. Hon, Y.-C. Chang and K.-P. Chu, *J. Chin. Chem. Soc.* (*Taipei*), **47**, 2000, 91.
98. B. Alcaide, P. Almendros and C. Aragoncillo, *Chem. Commun.* (*Cambridge*), 2000, 757.
99. F.-X. Felpin, E. Doris, A. Wagner, A. Valleix, B. Rousseau and C. Mioskowski, *J. Org. Chem.*, **66**, 2001, 305.
100. G.-H. Jeon, J.-Y. Yoon, S. Kim and S. S. Kim, *Synlett*, **1**, 2000, 128.
101. L. Sbaragli, F. Russo, M. Valacchi, G. Reginato and A. Mordini *Proc ECSOC*-3; *Proc. ECSOC*-4, 1999–2000, 659; *Chem. Abs.*, **132**,133579.
102. J. C. Anderson and A. Flaherty, *J. Chem. Soc., Perkin Trans. 2*, 2001, 267.
103. L. Wei and W. D. Lubell, *Can. J. Chem.*, **79**, 2001, 94.
104. G. Guichard, V. Semetey, C. Didierjean, A. Aubry, J.-P. Briand and M. Rodriguez, *J. Org. Chem.*, **64**, 1999, 8702.
105. M. Beller and M. Eckert, *Angew. Chem., Int. Ed.*, **39**, 2000, 1010.
106. M. P. Sibi and P. K. Deshpande, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1461.
107. S. Nomoto and A. Shimoyama, *Tetrahedron Lett.*, **42**, 2001, 1753.
108. K. Oertel, G. Zech and H. Kunz, *Angew. Chem. Int. Ed.*, **39**, 2000, 1431.
109. A. Gaucher, M. Wakselman, J.-P. Mazaleyrat, M. Crisma. F. Formaggio and C. Toniolo, *Tetrahedron*, **56**, 2000, 1715.
110. M. W. M. Tamm, T. Labahn, M. Noltemeyer, M. Es-Sayed and A. de Meijere, *J. Org. Chem.*, **65**, 2000, 3850.
111. G. D. Monache, D. Misiti, P. Salvatore and G. Zappia, *Tetrahedron: Asymmetry*, **11**, 2000,1137.
112. F. Wei, J.-B. Ni and M. Xian, *Huadog Ligong Daxue Xuebao*, **26**, 2000, 110; *Chem. Abs.*, **132**, 289718.
113. S. Abele and D. Seebach, *Eur. J. Org.*, **1**, 2000, 1.
114. K. Weber, S. Kuklinski and P. Gmeiner, *Org. Lett.*, **2**, 2000, 647.
115. S.-C. Sheng and Z.-S. Zhu, *Youji Huaxue*, **20**, 2000, 577; *Chem. Abs.*, **133**, 322111.
116. V. A. Soloshonok, C. Cai and V. J. Hruby, *Org. Lett.*, **2**, 2000, 747.
117. V. A. Soloshonok, C. Cai and V. J. Hruby, *Angew. Chem., Int. Ed.*, **39**, 2000, 2172.
118. B. Sauvagnat, F. Laaty, R. Lazaro and J. Martinez, *Tetrahedron Lett.*, **41**, 2000, 6371.
119. X. Tang, V. A. Soloshonok and V. A. Hruby, *Tetrahedron: Asymmetry*, **11**, 2000, 2917.

120. C. Agami, S. Comesse and C. Kadouri-Puchot, *J. Org. Chem.*, **65**, 2000, 4435.
121. S. Caddick, N. J. Parr and M. C. Pritchard, *Tetrahedron Lett.*, **41**, 2000, 5963.
122. R. Mazurkiewicz and M. Grymel, *Phosphorus, Sulfur Silicon Relat. Elem.*, **164**, 2000, 33.
123. R. Chinchilla, L. R. Falvello, N. Galindo and C. Najera, *J. Org. Chem.*, **65**, 2000, 3034.
124. T. Abellan, R. Chinchilla, N. Galindo, G. Guillena, C. Najera and J. M. Sansano, *Eur. J. Org. Chem.*, 2000, 2689.
125. T. Huang, C.-J. Li, *Tetrahedron Lett.*, **41**, 2000, 6715.
126. G. Borg, M. Chino and J. A. Ellman, *Tetrahedron Lett.*, **42**, 2001, 1433.
127. E. Juaristi, M. Balderas, J. L. Leon-Romo, H. Lopez-Ruiz and A. Reyes, *Curr. Trends Org. Synth.*, 1999, 199.
128. C. Najera, T. Abellan and J. M. Sansano, *Eur. J. Org. Chem.*, 2000, 2809.
129. H. Migabe, C. Ushiro, M. Ueda, K. Yamakawa and T. Naito, *J. Org. Chem.*, **65**, 1999, 176.
130. P. M. T. Ferreira, H. S. L. Maia, L. S. Monteiro and J. Sacramento, *Tetrahedron Lett.*, **41**, 2000, 7437.
131. S. Varray, C. Gauzy, F. Lamaty, R. Lazaro and J. Martinez, *J. Org. Chem.*, **65**, 2000, 6787.
132. H. J. C. Deboves, U. Grabowska, A. Rizzo and R. F. W. Jackson, *J. Chem. Soc., Perkin Trans. 1*, 2000, 4284.
133. A. Ohashi and T. Imamoto, *Org. Lett.*, **3**, 2001, 373.
134. P. Li, *Hebei Gongye Keji*, **17**, 2000, 45; *Chem. Abs.*, **132**, 703576.
135. S. -Y. Zhang, W.-H. Guo and D.-J. Wu, *Hecheng Huaxue*, **8**, 2000, 107; *Chem. Abs.*, **133** 105271.
136. C. E. Masse, A. J. Morgan, J. Adams and J. S. Panek, *Eur. J. Org. Chem.*, 2000, 2513.
137. S. Sano and Y. Nagao, *Yuki Gosei Kagaku Kyokaishi*, **58**, 2000, 756; *Chem. Abs.*, **133**, 177427.
138. H. Sato, *Kirara Tekunoroji no Kogyoka*, 1998, 112; *Chem. Abs.* **133**, 609.
139. W. Wang, *Niangjiu*, 2000, 30.
140. H. Xu and J. Xu, *Huaxue Shijie*, **41**, 2000, 383.
141. C.-H. Cheng and S.-W. Tsai, *J. Chin. Inst. Chem. Eng.*, **31**, 2000, 177.
142. T. Ooi, M. Kameda, H. Tannai and K. Maruoka, *Tetrahedron Lett.*, **41**, 2000, 8339.
143. Y. Fang, W. Zhang, S. Yang Q. Lu and X. Ding, *Guangzhou Huagong*, **28**, 2000, 134, *Chem. Abs.*, 2001, 109046.
144. B. Zhou and Y. Fang, *Guangzhou Huagong*, **28**, 2000, 85; *Chem. Abs.*, **132**, 109029.
145. V. P. Shevchenko, I. Y. Nagaev and N. F. Myasoedov, *Radiochemistry* (*Moscow*), **42**, 2000, 188.
146. F. Hanic, M. Morvova and I. Morva, *J. Therm Anal. Calorim.*, **60**, 2000, 1111.
147. L. Beauge, M. Siravegna and G. Berberian, *Anal. Biochem.*, **286**, 2000, 306.
148. L. Andersen, B. Nielsen and J. W. Jaroszewski, *Chirality*, **12**, 2000, 665.
149. P.Wei and W.Wu, *J. Chin. Pharm. Sci.*, **8**, 1999, 212.
150. J. Jemielity, M. Kanska and R. Kanski, *Isot. Environ. Health Stud.*, **34**, 1998, 335.
151. H. Maeda, S.-I. Suzuki, M. Ikeguchi, T. Sakai and K. Shibata, *J. Biosci. Bioeng.*, **89**, 2000, 392.
152. A. Ogrel, V. I. Shvets, B. Kaptein, Q. B. Broxterman and J. Raap, *Eur. J. Org. Chem.*, **5**, 2000, 857.
153. Z.-K. Duan, Y.-Q. Yang, W.-Y. Liu, Y. Xiong and G.-l. Li, *Jiingxi Huagong*, **17**, 2000, 345.
154. S. Xiang, C. Zhao, J. Wen and Z. Zhu, *Huaxue Shijie*, **41**, 2000, 30; *Chem. Abs.*, **133**

43762.
155. A. J. Minnaard, W. H. J. Boesten and H. J. M. Zeegers, *Synth. Commun.*, **29**, 1999, 4327.
156. G. T. M. Titulaer, J. Zhu, A. J. H. Klunder and B. Zwanenburg, *Org. Lett.*, **2**, 2000, 473.
157. A. Reyes and E. Juaristi, *Tetrahedron: Asymmetry*, **11**, 2000, 1411.
158. L.-k. Zhang, X.-R. Chen and Y. Huang, *Sichuan Lianhe Daxue Xuebao Gongcheng Kexueban*, **3**, 1999, 530.
159. J. P. Nandi, B. Saha, S. Sharma, S. Bose and J. Iqbal, *J. Indian Chem. Soc.*, 76, 1999, 719.
160. S. Kotha, S. Herve, E. Bramachary and T. Ganesh, *Synlett*, 2000, 896.
161. Q. Wu, G. Wang, X. Wang, T. Wu, A. S. C. Chan and T.-K. Yang, *Tetrahedron: Asymmetry*, **11**, 2000, 2309.
162. J. T. Kendall, *J. Labelled Compd. Radiopharm.*, **43**, 2000, 505.
163. J. T. Kendall, *J. Labelled Compd. Radiopharm.*, **43**, 2000, 917.
164. M. Adamczyk, J. R. Fishpaugh and M. Thiruvazhi, *J. Labelled Compd. Radiopharm.*, **43**, 2000, 909.
165. S.-B. Chen, Y.-M. Li, S.-Zhong Luo, G. Zhao, B. Tan and Y.-F. Zhao, *Phosphorus, Sulfur Silicon Relat. Elem.*, **164**, 2000, 277.
166. A. Dong, X. Zhang, Z. Li and H. Liu, *Huaxue Shijie*, **42**, 2001, 33, *Chem. Abs.* 2001, 126950.
167. D. A. Pearce, A. M. Sargeson, A. Hammershoi and J. M. Harrowfield, *Chem. Commun.* (*Cambridge*), 2000, 2431.
168. F. Jeanjean, G. Fournet, D. Le Bars and J. Gore, *Eur. J. Org. Chem.*, 2000, 1297.
169. J. R. Harding, R. A. Hughes, N. M. Kelly, A. Sutherland and C. L. Wilis, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3406.
170. S. Kobayashi, R. Akiyama and H. Kitagawa, *J. Comb. Chem.*, **2**, 2000, 438.
171. S. Boukhris and A. Souizi, *Tetrahedron Lett.*, **41**, 2000, 2559.
172. N. A. Sasaki, *Methods Mol. Med.*, 1999, 23.
173. N. Langlois and F. Rakotondradany, *Tetrahedron*, **56**, 2000, 2437.
174. M. Garcia, A. Serra, M. Rubiralta, A. Diez, V. Segarra, E. Lozoya, H. Ryder and J. M. Palacios, *Tetrahedron: Asymmetry*, **11**, 2000, 991.
175. G. Veeresa, *Synth. Commun.*, **30**, 2000, 1479.
176. M. Sabat and C. R. Johnson, *Tetrahedron Lett.*, **42**, 2001, 1209.
177. J. De Blas, E. Dominguez and J. Ezquerra, *Tetrahedron Lett.*, **41**, 2000, 4567.
178. P. Allevi and M. Anastasia, *Tetrahedron: Asymmetry*, **11**, 2000, 3151.
179. A. D. Campbell, T. M. Raynham and R. J. K. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3194.
180. O. Miyata, Y. Ozawa, I. Ninomiya and T. Naito, *Tetrahedron*, **56**, 2000, 6199.
181. H. Nakagawa, T Sugahara and K. Ogasawara, *Org. Lett.*, **2**, 2000, 3181.
182. J. E. Baldwin, G. J. Pritchard and D. S. Williamson, *Biorg. Med. Chem. Lett.*, **10**, 2000, 1927.
183. A. Hercouet, M. Le Corre and B. Carboni, *Tetrahedron Lett.*, **41**, 2000, 197.
184. C. Belzecki, J. Trojnar and M. Chmielewski, *Synth. Commun.*, **30**, 2000, 2245.
185. T. Shiraiwa, K. Nakagawa and N. Korimoto, *Chem. Lett.*, 2000, 468.
186. M. J. Milewska, *Pol. J. Chem.*, **74**, 2000, 447.
187. C. Ma, S. Yu, X. Liu and J. M. Cook, *Tetrahedron Lett.*, **41**, 2000, 2781.
188. M. Baenziger, L. Gobbi, B. P. Riss, F. Schaefer and A. Vaupel, *Tetrahedron: Asymmetry*, **11**, 2000, 2195.
189. M. C. Pirrung and T. Pei, *J. Org. Chem.*, **65**, 2000, 2229.

190. W. Li and X. Wang, *Huagong Shikan*, **13**, 1999, 16.
191. J.-L. Zhao, Y. Zhang, B.-Q. Cui, D.-L. Jiang, D.-P. Gu, *Hebei Shifan Daxue Xuebao*, *Ziran Kexueban*, **24**, 2000, 360; *Chem. Abs*., 2000, 811021.
192. S. Sinha, S. Tilve and S. Chandrasekaran, *Proc. ECSOC*-3, *Proc. ECSOC*-4, 1999–2000 636; *Chem. Abs*., 2001, 133571.
193. B. Liang, P. J. Carroll and M. M. Joullie, *Org. Lett*., **2**, 2000, 4157.
194. R. E. Dolle, T. F. Herpin, and Y. C. Shimshock, *Tetrahedron Lett*., **42**, 2001, 1855.
195. C. T. Supuran, F. Briganti, G. Mincione and A. Scozzafava, *J. Enzyme Inhib*., **15**, 2000, 111.
196. A. Scozzafava and C. T. Supuran, *Bioorg. Med. Chem. Lett*., **10**, 2000, 499.
197. P. Merino, S. Anoro, S. Franco, F. l. Merchan, T. Tejero and V. Tunon, *J. Org. Chem*., **65**, 2000, 1590.
198. J. M. Andres, N. De Elena and R. Pedrosa, *Tetrahedron*, **56**, 2000, 1523.
199. M. D. Fletcher, J. R. Harding, R. A. Hughes, N. M. Kelly, H. Schmalz, A. Sutherland and C. L. Willis, *J. Chem. Soc*., *Perkin Trans. 1*, 2000, 43.
200. A. O. Martirosyan, S. P. Gasparyan, V. E. Oganesyan, S. L. Mndzhoyan, M. V. Aleksanyan, M. N. Nikishchenko and G. S. Babayan, *Chem. Hetercycl. Compd. (N Y)*, **36**, 2000, 416.
201. C. Dittmar, *Ber. Forschungszent. Juelich*, Juel-3766, I-vi, 2000, 1; *Chem. Abs*., 2000, 710016.
202. J.-L. Zhao, D.-L. Jiang, D.-P. Gu, Y. Zhang, B.-Q. Cui, *Hebei Shifan Daxue Xuebao*, *Ziran Kexueban*, **24**, 2000, 216; *Chem. Abs.* 2000, 630410.
203. S. Yue, *Huaxue Yanjiu Yu Yingyong*, **12**, 2000, 387; *Chem. Abs*., 2000, 704426.
204. S. Mzengeza, T. K. Venkatachalam and M. Diksic, *Amino Acids*, **18**, 2000, 81.
205. A. V. Soloshonok, A. Vadim, X. Tang, V. J. Hruby and L. Van Meervelt, *Org. Lett*., **3**, 2001, 341.
206. A. J. Minnaard, W. H. J. Boesten and H. J. M. Zeegers, *Synth. Commun*., **29**, 1999, 4327.
207. A. Singh, A. K. Prasad, W. Errington, Y. N. Belokon, K. A. Kochetkov, R. K. Saxena, S. C. Jain and V. S. Parmar, *Indian J. Chem*., *Sect. B*, *Incl. Med. Chem*., **39B**, 2000, 10.
208. B. Poojary S. L. Belagali, K. H. Kumar, B. S. Holla and R. Gonsalves, *Indian J. Heterocycl. Chem*., **9**, 2000, 263.
209. H. Uno, K.-I. Kasahara and N. Ono, *Heterocycles*, **53**, 2000, 1011.
210. F. Fulop, *Stud. Nat. Prod. Chem*. (*Bioactive Natural Products*, *Part C*), **22**, 2000, 273.
211. T. Yamada, D. Tsuboi, K. Okano, A. Kanda, Y. Nishi, F. Yamanishi, Y. Origani, Y. Nakamura, R. Yanagihara and T. Miyazawa, *Pept. Sci*., 36*th*, 1999, 147.
212. F. A. Davis, S. Lee, H. Zhang, D. L. Faneli, *J. Org. Chem*., **65**, 2000, 8707.
213. D. Ma and K. Ding, *Org. Lett*., **2**, 2000, 2515.
214. H. Sasaki and E.M.Carreira, *Synthesis*, **1**, 2000, 135.
215. S. Carbonnel, C. Fayet, J. Gelas and Y. Troin, *Tetrahedron Lett*., **41**, 2000, 8293.
216. F. A. Davis, T. Fang, B. Chao and D. M. Burns, *Synthesis*, 2000, 2106.
217. A. J. Souers and J. A. Ellman, *J. Org. Chem*., **65**, 2000, 1222.
218. Y. Matsumura, M. Inoue, Y. Nakamura, I. L. Talib, T. Maki and O. Onamura, *Tetrahedron Lett*., **41**, 2000, 4619.
219. J. Wede, F.-J. Volk and A. W. Frahm, *Tetrahedron*: *Asymmetry*, **11**, 2000, 3231.
220. O. Schrake, V. S. Rahn, J. Frackenpohl, W. E. M. Braje and H. M. R. Hoffmann, *Org. Lett*., **1**, 1999, 1607.
221. A. Avenoza, J. H. Busto, C. Cativiela and J. M. Peregrina, *Amino Acids*, **18**, 2000, 117.

222. T. Abelland, C. Najera and J. M. Sansano, *Tetrahedron*: *Asymmetry*, **11**, 2000, 1051.
223. D. R. Zitsane, I. T. Ravinya, I. A. Riikure, Z. F. Tetere, E. Y. Gudrinietse and U. O. Kalei, *Russ. J. Org. Chem.*, **35**, 1999, 1457.
224. L. Halab, F. Gosselin and W. D. Lubell, *Biopolymers*, **55**, 2000, 101.
225. D. H. Appella, P. R. LePlae, T. L. Raguse and S. H. Gellman, *J. Org. Chem.*, **65**, 2000, 4766.
226. Y. Nishiyama, S. Ishizuka, S. Shikama and K. Kurita, *Pept. Sci.*, 36*th*, 1999, 91.
227. J. Rife, R. M. Ortuno and G. A. Lajoie, *J. Org. Chem.*, **64**, 1999, 8958.
228. D. Ma, Y. Cao, W. Wu and Y. Jiang, *Tetrahedron*, **56**, 2000, 7447.
229. M. Marinozzi and R. Pellicciari, *Tetrahedron Lett.*, **41**, 2000, 9113.
230. J. Wichmann, P. Huguenin and G. Adam, *J. Labelled Compd. Radiopharm.*, **43**, 2000, 1.
231. M. Sasaki, T. Koike, R. Sakai and K. Tachibana, *Tetrahedron Lett.*, 41, 2000, 3923.
232. H. Masaki, J. Maeyama, K. Kamada, T. Esumi, Y. Iwabuchi and S. Hatakeyama, *J. Am. Chem. Soc.*, **122**, 2000, 5216.
233. C. A. Brooks and D. L. Comins, *Tetrahedron Lett.*, **41**, 2000, 3551.
234. A. G. Moglioni, E. Garcia-Exposito, G. P. Aguado, T. Parella, V. Branchadell, G. Y. Moltrasio and R. M. Ortuno, *J. Org. Chem.*, **65**, 2000, 3934.
235. A. Krasinski and J. Jurczak, *Tetrahedron Lett.*, **42**, 2001, 2019.
236. K. Hammer, J. Wang, M. L. Falck-Pedersen, C. Romming and K. Undheim, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1691.
237. J. Ogawa, A. Ryono, S.-X. Xie, R. M. Vohra, R. Indrati, M. Akamatsu, H. Miyagawa, T. Ueno and S. Shimizu, *J. Mol. Catal. B*: *Enzym.*, **12**, 2001, 71.
238. A. Avenoza, C. Cativiela, M. A. Fernandez-Reico, A. Miguel and J. M. Peregrina, *J. Chem. Soc., Perkin Trans. 2*, 1999, 3375.
239. C. Pedregal, I. Collado, A. Escribano, J. Ezquerra, C. Dominguez, A. I. Mateo, A. Rubio, S. R. Baker, J. Goldsworthy, R. K. Kamboj, B. A. Ballyk, K. Hoo and D. Bleakman, *J. Med. Chem.*, **43**, 2000, 1958.
240. Y. Gao and T. R. Burke Jr., *Synlett*, **1**, 2000, 134.
241. F. Clerici, M. L. Gelmi and A. Gambini, *J. Org. Chem.*, **65**, 2000, 6138.
242. I. O. Donkor, X. Zheng, J. Han and D. D. Miller, *Chirality*, **12**, 2000, 551.
243. G. Suter, S. Stoykova, A. Linden and H. Heimgartner, *Helv. Chim. Acta*, **83**, 2000, 2961.
244. Y. Y. Kozyrkov, A. Pukin, O. G. Kulinkovich, J. Ollivier and J. Salaun, *Tetrahedron Lett.*, **41**, 2000 6399.
245. S. R. Baker, K. Goodall, A. F. Parson, and M. Wilson, *J. Chem. Res., Synop.*, **312-313**, 2000, 837.
246. S. Kotha, T. Ganesh and A. K. Ghosh, *Bioorg. Med. Chem. Lett.*, **10**, 2000, 1755.
247. S. N. Osipov, O. I. Artyushin, A. F. Kolomiets, C. Bruneau and P. H. Dixneuf, *Synlett*, 2000, 1031.
248. D. Zicane, I. Ravina, Z. Tetere, I. Rijkure and E. Gudriniece, *Materialzinat, Lietiska Kim.*, **1**, 2000, 142.
249. M. J. Coleman, M. D. Goodyear, D. W. S. Latham and A. J. Whitehead, *Synlett*, **12**, 1999, 1923.
250. K. M. Depew, M. Theodore and S. J. Danishefsky, *Tetrahedron Lett.*, **41**, 2000, 289.
251. P. Karfarski and J. Zon, *Aminophosphonic Aminophosphinic Acids*, 2000, 33.
252. I. G. C. Coutts, R. E. Saint and L. S. Saint, *Synthesis*, **2**, 2001, 247.
253. S. Lochynski, J. Kuldo, B. Frakowiak, J. Holband and G. Wojcik, *Tetrahedron*: *Asymmetry*, **11**, 2000, 1295.
254. R. Arnaud, C. Adamo, M. Cossi, A. Milet, Y. Vallée and V. Barone, *J. Am. Chem.*

Soc., **122**, 2000, 324.
255. K. Kobayashi, H. Masuda, K. Ushio, T. Kasamatsu, T. Kaneko, J. Takahashi, T. Hosokawa and T. Saito, *Tokyo Daigaku Genshiryoku Kenkyu Sogo Senta Shinpojumu 8th*, 1999, 70; *Chem. Abs*., 2001, 156009.
256. D. Trancard, J.-B. Tout, T. Giard, I. Chichaoui, D. Cahard and J.-C. Plaquevent, *Tetrahedron Lett*., **41**, 2000, 3843.
257. T. Lindel, M. Hochguertel, M. Assmann and M. Koeck, *J. Nat. Prod*., **63**, 2000, 1566.
258. K. Uneyama, *Enantiocontrolled Synth. Fluoro-Org. Compd*., 1999, 391.
259. A. Sutherland and C. L. Willis, *Nat. Prod. Rep*., **14**, 1997, 205.
260. A. Sutherland and C. L. Willis, *Nat. Prod. Rep*., **17**, 2000, 621.
261. I. I. Gerus, A. A. Kolomeitsev, M. I. Kolycheva and V. P. Kukhar, *J. Fluorine Chem*., **105**, 2000, 31.
262. M. Mae, H. Amii and K. Uneyama, *Tetrahedron Lett*., **41**, 2000, 7893.
263. S. M. Mazza, *J. Labelled Compd. Radiopharm*., **43**, 2000, 1047.
264. S. N. Osipov and K. Burger, *Tetrahedron Lett*., **41**, 2000, 5659.
265. M. Crucianelli, N. Battista, P. Bravo, A. Volonterio and M. Zanda, *Proc. ECSOC*-3, 1999–2000, 704.
266. M. Moroni, B. Koksch, S. N. Osipov, M. Crucianelli, M. Frigerio, P. Bravo and K. Burger, *J. Org. Chem*., **66**, 2001, 130.
267. M. Hasegawa, K. Yamada and Y. Nagahama, *Heterocycles*, **51**, 1999, 2815.
268. H. Mues and U. Kazmaier, *Synlett*, **7**, 2000 1031.
269. F. A. Davis, V. Srirajan, D. L. Fanelli and P. Portonono, *J. Org. Chem*., **65**, 2000, 7663.
270. M. Fujii, T. Miura, T. Kajimoto and Y. Ida, *Synlett*, **7**, 2000, 1046.
271. D. L. Boger, R. J. Lee, P.-Y. Bounaud and P. Meier, *J. Org. Chem*., **65**, 2000, 6770.
272. K. Shimamoto, Y. Shigeri, Y. Yasuda-Kamatani, B. Lebrun, N. Yumoto and T. Nakajima, *Bioorg. Med. Chem. Lett*., **10**, 2000, 2407.
273. F. A. Davies, H. Zhang and S. H. Lee, *Org. Lett*., **3**, 2001, 759.
274. X. Lin, H. Dorr and J. M. Nuss, *Tetrahedron Lett*., **41**, 2000, 3309.
275. L. Aurelio, R. T. C. Brownlee, A. B. Hughes and B. E. Sleebs, *Aust. J. Chem*., **53**, 2000, 425.
276. A. Detomaso and R. Curci, *Tetrahedron Lett*., **42**, 2001, 755.
277. B. M. Kim, J. S. Park and J. H. Cho, *Tetrahedron Lett*., **41**, 2000, 10031.
278. F.-R. Wang, B.-l. Gong, X.-Q. Li, L.-F. Wang and Q.-H. Chen, *Ningxia Daxue Xuedao Ziran Kexueban*, **21**, 2000, 342.
279. Y. Song, A. D. Sercel, D. R. Johnson, N. L. Colbry, K.-L. Sun and B. D. Roth, *Tetrahedron Lett*., **41**, 2000, 8225.
280. J. Staukas, N. Dirvianskyte and E. Butkus, *J. Prakt. Chem.* (*Weinheim, Ger*.), **342**, 2000, 715.
281. T. Xu, X. Jiang, P. Ni, W. Hua and Y. Pei, *Yaoxue Xuebao*, **35**, 2000, 228; *Chem. Abs*., **133**, 164279.
282. F. A. Luzzio, A. V. Mayorov and W. D. Figg, *Tetrahedron Lett*., **41**, 2000, 2275.
283. A. N. Popkov, *Chem. Heterocycl. Compd*. (*N Y*), **36**, 2000, 544.
284. V. K. Aggarwal, J. K. Barrell, J. M. Worrall and R. Alexander, *Phosphororus, Sulfur Silicon Relat. Elem*., 1999, 153.
285. D. Kong, Q. Chen, Y. Xie and X. Zhou, *Zhongguo Yaowu Huaxue Zazhi*, **10**, 2000, 13.
286. K. Nagren, E. Sutinen and S. Jyrkkio, *J. Labelled Compd. Radiopharm*., **43**, 2000, 1013.

287. M. A. Massa, B. S. Pitzele, G. M. Jerome, W. M. Moore, P. T. Manning and J. A. Sikorski, *Heteroat. Chem.*, **11**, 2000, 505.
288. S. J. Chung and D. H. Kim, *Bioorg. Med. Chem.*, **9**, 2000, 185.
289. A. N. Sabirov and V.V. Samukov, *Pept.* 1998, *Proc. Eur Pept. Symp.*, 25*th*, 1999, 112.
290. J. G. Wilson, and A. G. Katsifis, *Aust. J. Chem.*, **53**, 2000, 583.
291. B. S. Jursic, D. Neumann and A. McPherson, *Synthesis*, 2000, 1656.
292. H. Xu, Q. Zhang, X.-J. Kong, *Jingxi Huagong*, **17**, 2000, 205; *Chem. Abs.*, **133**, 323264.
293. J. Wang, Y. Zhao and T. Wang, *Zhongguo Yaowu Huaxue Zazhi*, **10**, 2000, 283.
294. B. Henkel and E. Bayer, *Synthesis*, 2000, 1211.
295. E. Medina, A. Moyano, M. A. Pericas and A. Riera, *Helv. Chim. Acta*, **83**, 2000, 972.
296. Y. Ueda, H. Manabe, M. Mitsuda and M. Kitamura, *Chem. Eng. Res. Des.*, **78**, 2000, 756.
297. P. Dauban, C. De Saint-Fuscien, F. Acher, L. Prezeau, I. Brabet, J.-P. Pin and R. H. Dodd, *Bioorg. Med. Chem. Lett.*, **10**, 2000, 129.
298. L. Demange, J. Cluzeau, A. Menez and C. Dugave, *Tetrahedron Lett.*, **42**, 2001, 651.
299. A. Pinazo, M. R. Infante, P. Izquierdo and C. Solans, *J. Chem. Soc., Perkin Trans. 2*, 2000, 1535.
300. P. Liu, J. B. Chang, R. F. Chen, Q. Wang and J. X. Xie, *Chin. Chem. Lett.*, **11**, 2000, 391.
301. J. Rudolph, *Tetrahedron*, **56**, 2000, 3161.
302. T. R. Burke, D.-G. Liu and Y. Gao, *J. Org. Chem.*, **65**, 2000, 6288.
303. A. M. Shalaby, M. H. Abo-Ghalia and H. M. Awad, *Modell. Meas. Control, C*, **58**, 1999, 9.
304. V. Mickevicius, Z. Beresnevicius and E. Jakiene, *Biologija*, **1**, 1999, 29.
305. H. Nemeto, R. Ma, I. Suzuki and M. Shibuya, *Org. Lett.*, **2**, 2000, 4245.
306. K. Burger and J. Spengler, *Eur. J. Org. Chem.*, **1**, 2000, 199.
307. H. Lu, K. L. Mlodnosky, T. T. Dinh, A. Dastgah, V. Q. Lam and C. E. Berkman, *J. Org. Chem.*, **64**, 1999, 8698.
308. C. Peggion, R. Flammengo, E. Mossel, Q. B. Broxterman, B. Kaptein, J. Kamphius, F. Formaggio, M. Crisma and C. Toniolo, *Tetrahedron*, **56**, 2000, 3589.
309. T. Nagano and H. Kinoshita, *Bull. Chem. Soc. Jpn.*, **73**, 2000, 1605.
310. S. R. Baker, D. Bleakman, J. Ezquerra, B. A. Ballyk, M. Deverill, K. Ho, R. K. Kamboj, I. Collado, C. Dominguez, A. Escribano, A. I. Mateo, C. Pedregal and A. Rubio, *Bioorg. Med. Chem. Lett.*, **10**, 2000, 1807.
311. H. Mues and U. Kazmaier, *Proc. ECSOC*-3; *Proc. ECSOC*-4, 1999–2000, 314.
312. Y. Yonezawa, T. Hirosaki, T. Hayashi and C.-G. Shin, *Synthesis*, **1**, 2000, 144.
313. S. Suzen and J. M. Williams, *Turk. J. Chem.*, **24**, 2000, 361.
314. K. Goodal and A. F. Parsons, *J. Chem. Res., Synop.*, **54-54**, 2000, 312.
315. D. B. Berkowitz, J. M. McFaddon, M. K. Smith and M. L. Pedersen, *Methods Mol. Med.*, 1999, 467.
316. J. M. McFadden, *Diss. Abstr. Int. B* 2000, **61**, 2000, 1956.
317. D. B. Berkowitz, J. M. McFadden, M. Jill, E. Chisowa and C. L. Semerad, *J. Am. Chem. Soc.*, **122**, 2000, 11031.
318. D. B. Berkowitz, J. M. McFadden and M. K. Sloss, *J. Org. Chem.*, **65**, 2000, 2907.
319. N. G. W. Rose, M. A. Blaskovich, A. Wong and G. A. Lajoie, *Tetrahedron*, **57**, 2001, 1497.
320. Y. Yonezawa, K. Shimizu, K.-S. Yoon and C.-G. Shin, *Synthesis*, 2000, 634.
321. C. Alvarez-Ibarra, A. G. Csaky and C. Gomez de la Oliva, *J. Org. Chem.*, **65**, 2000, 542.

322. Y.-N. Xie, X. Cui and A.-Q. Mi, Y.-Z. *Jiang Hecheng*, *Huaxue*, **8**, 2000, 33.
323. U. Kazmaier, D. Achlauss, M. Pohlman and S. Raddatz, *Synthesis*, 2000, 914.
324. D. J. Bennett, N. M. Hamilton, *Tetrahedron Lett*., **41**, 2000, 7961.
325. L. Vranicar, S. Polanc and M. Kocevar, *Zb. Ref. Posvetovanja Slov. Kem. Dnevi*, **288**, 1999, *Chem. Abs*., 1999: 788593.
326. G. Zvilichovsky and V. Gurvich, *Recent Dev. Org. Chem*., **3**, 1999, 87.
327. S. Liao and V. J. Hruby, *Methods Mol. Med*., 1999, 175.
328. D. Tourwe, K. Iterbeke, W. M. Kazmierski and G. Toth, *Methods Mol. Med*., 1999, 321.
329. S. Saaby, X. Fang, N. Gethergood and K. A. Jorgensen, *Angew. Chem. Int. Ed*., **39**, 2000, 4114.
330. N. Harada, S. Nishiyama, K. Sato and H. Tsukada, *Appl. Radiat. Isot*., **52**, 2000, 845.
331. S. Kotha and E. Brahmachary, *J. Org. Chem*., **65**, 2000, 1359.
332. P. S. Herradura, K. A. Pendola and R. K. Guy, *Org. Lett*., **2**, 2000, 2019.
333. P. N. Collier, A. D. Campbell, I. Patel and J. R. K. Taylor, *Tetrahedron Lett*., **41**, 2000, 7115.
334. H. Nemeto, R. Ma, H. Moriguchi, I. Suzuki and M. Shibuya, *J. Organomet. Chem*., **611**, 2000, 445.
335. P. Phukan, and A. Sudalai, *Indian J. Chem*., *Sect. B*: *Org. Chem. Incl. Med. Chem*., **39B**, 2000, 291.
336. M. Calmes, F. Escale, C. Glot, M. Rolland and J. Martinez, *Eur. J. Org. Chem*., 2000, 2459.
337. M. Sabat and C. R. Johnson, *Org. Lett*., **2**, 2000,1089.
338. W. Qiu, V. A. Soloshonok, C. Cai, X. Tang and V. J. Hruby, *Tetrahedron*, **56**, 2000, 2577.
339. Y. Han, A. Bisello, C. Nakamoto, M. Rosenblatt and M. Chorev, *J. Pept. Res*., **55**, 2000, 262.
340. E. Morera and G. Ortar, *Bioorg. Med. Chem. Lett*., **10**, 2000, 1815.
341. A. Avenoza, C. Cativiela, F. Corzana, J. M. Peregrina and M. M. Zurbano, *Tetrahedron*: *Asymmetry*, **11**, 2000, 2195.
342. R. M. Adlington, J. E. Baldwin. D. Catterick, G. J. Pritchard and L. T. Tang, *J. Chem. Soc*., *Perkin Trans. 1*, 2000, 2311.
343. M. Skof, J. Svete, B. Stanovnik and S. Grddadolnik-Golic, *Acta Chim. Slov*., **46**, 1999, 567.
344. N. Haroune, M. L. Roummestant and J. Martinez, *Tetrahedron*: *Asymmetry*, **11**, 2000, 4719.
345. G. Cabarrocas, S. Rafel, M. Ventura and J. M. Villalgordo, *Synlett*, 2000, 595.
346. M. Onishi and H. Tamiaki, *Pept. Sci*., 36*th*, 1999, 357.
347. G. Leidl, H.-J. Musiol, N. Budisa, R. Huber, S. Poiot, D. Fourmy and L. Moroder, *J. Pept. Sci*., **6**, 2000, 139.
348. L. De Luca, G. Giacomelli, A. Porcheddu, A. M. Spanedda and M. Falorni, *Synthesis*, 2000, 1295.
349. M. Polak and B. Vercek, *Synth. Commun*., **30**, 2000, 2863.
350. R. M. Adlington, J. E. Baldwin, D. Catterick and G. J. Pritchard, *J. Chem. Soc. Perkin Trans. 1*, 2000, 299.
351. M. Hasan, F. Shaheen, U. Yunus and S. Munir, *Turk. J. Chem*., **24**, 2000, 165.
352. J. S. McMurray, O. Khabashesku, J. S. Birtwistle and W. Wang, *Tetrahedron Lett*., **41**, 2000, 6555.
353. R. C. F. Jones, D. J. C. Berthelot, J. N. Iley, *Chem. Commun*. (*Cambridge*), 2000,

2131.
354. M. Stanchev, S. Tabakova, G. Videnov, E. Golovinskiand and G. Jung, *Arch. Pharm.* (*Weinheim Ger.*), **332**, 1999, 297.
355. S.-M. Lu and B. Liu, *Huadong Shifan Daxue Xuebao Ziran Kexueban*, **2**, 2000, 107.
356. M. Stanchev, T. Pajpanova and E. Golovinsky, *Amino Acids*, **18**, 2000, 177.
357. R. C. F. Jones, S. J. Hollis and J. N. Hey, *Tetrahedron*: *Asymmetry*, **11**, 2000, 3273.
358. P. Conti, M. De Amici and C. De Micheli, *Farmaco*, **55**, 2000, 162.
359. F. Ikegami, A. Yamamoto, T. Sekine, T. Ishikawa, K. Kusamae-Eguchi, T. Kusama and K. Watanabe, *Chem. Pharm. Bull.*, **48**, 2000, 296.
360. P. J. Zimmermann, I. Blanarikova and V. Jager, *Angew. Chem., Int. Ed.*, **39**, 2000, 910.
361. U. Koert, *J. Prakt. Chem.*, **342**, 2000, 325.
362. D. Zicane, I. Ravina, Z. Tetere, I. Rijkure and E. Gudrinece, *Latv. Kim. Z.*, 2000, 62.
363. L. Lecointe, V. Rolland, L. Pappalardo, M. L. Roumestant, P. Viallefont and J. Martinez, *J. Pept. Res.*, **55**, 2000, 300.
364. A. Matsushima, T. Fujita, K. Okada, Y. Yamauchi, T. Nose and Y. Shimohigashi, *Pept. Sci.*, 36*th*, 1999, 137.
365. A. Scozzafava and C. T. Supuran, *J. Med. Chem.*, **35**, 2000, 299.
366. R. M. Adlington, J. E. Baldwin, D. Catterick, G. J. Pritchard and L. T. Tang *J. Chem. Soc. Perkin Trans. 1*, 2000, 303.
367. P. D. Croce, C. La Rosa, E. Pizzati, *Tetrahedron*: *Asymmetry*, **11**, 2000, 2635.
368. H. Nemeto, R. MA, H. Moriguchi, I. Suzuki, M. Shibuya, *J. Organomet. Chem.*, **611**, 2000, 445.
369. C. Basu, V. Sunel, C. Soldea and D. Maftei-Sirbu, '*Al. I. Cuza*' *Iasa*, *Chim.*, **6**, 1998, 51; *Chem. Abs.* 2000, 28758.
370. V. Sunel, C. Basu, C. Soldea and D. Maftei-Sirbu, '*Al. I. Cuza*' *Iasa*, *Chim.*, **6**, 1998, 35; *Chem. Abs.*, 2000, 28756.
371. M. Adamczyk, D. D. Johnson and R. E. Reddy, *Tetrahedron*: *Asymmetry*, **11**, 2000, 3063.
372. V. V. S. Babu, K. Ananda, G.-R. Vasanthakumar, *J. Chem. Soc., Perkin Trans. 1*, 2000, 4328.
373. K. L. Kirk, B. Herbert, S.-F. Lu, B. Jayachandran, W. L. Padgett, O. Olufunke, J. W. Daly, G. Haufe and L. K. Laue, *ACS Symp. Ser.*, 746 (*Asymmetric Fluoroorganic Chemistry*) 2000, 194.
374. A. Cheguillaume, I. Doubli-Bounoua, M. Baudy-Floch and P. Le Grel, *Synlett*, 2000, 331.
375. A. Cheguillaume, I. Doubli-Bounoua, M. Baudy-Floch and P. Le Grel, *Synlett*, 2000, 331.
376. T. S. Maurer, J. Pan, B. P. Booth, T. I. Calman and H.-L. Fung, *Biorg. Med. Chem. Lett.*, **10**, 2000,1077.
377. A. J. Robinson and D. Mulholland, *J. Org. Chem.*, **66**, 2001, 755.
378. M. Sypniewski, B. Penke, L. Simon and J. Rivier, *J. Org. Chem.*, **65**, 2000, 6595.
379. F. Paradisi, G. Porzi, S. Rinaldi and S. Sandri, *Tetrahedron*: *Asymmetry*, **11**, 2000, 1259.
380. D. D. Hennings and R. M. Williams, *Synthesis*, 2000, 1310.
381. K.-I. Tanaka and H. Sawanishi, *Tetrahedron*: *Asymmetry*, **11**, 2000, 920.
382. K. Burger, J. Spengler, L. Hennig, R. Herzschuh and S. A. Essawy, *Monatsh. Chem.*, **131**, 2000, 463.
383. S. Collet, F. Carreaux, J.-L. Boucher, S. Pethe, M. Lepoivre, R. Danion-Bougot and D. Danion, *J. Chem. Soc., Perkins Trans. 2*, 2000, 177.

384. G. W. Kabalka, N. K. Reddy, L. Wang and R. R. Malladi, *Org. Prep. Proced. Int*., **32**, 2000, 290.
385. M. Kimura, K. Yoshimo and H. Tomiyashu, *Proc. Int. Conf. Future Nucl. Syst*., 1999, 1598; *Chem. Abs*., 2000, 596157,
386. C. K. Park, K. Yoshino and H. Tomiyasu, *Synthesis*, **12**, 1999, 2041.
387. H. Nakamura, M. Fujiwara and Y. Yamamoto, *Bull. Chem. Soc. Jpn*., **73**, 2000, 231.
388. B. K. Shull, D. E. Spielvogel, G. Head, R. Gopalaswamy, S. Sankar and K. Devito, *J. Pharm. Sci*., **89**, 2000, 215.
389. R. Tacke, M. Merget, R. Bertermann, M. Bernd, T. Beckers and T. Reissmann, *Organometallics*, **19**, 2000, 3486.
390. M. Merget, S. Bartoschek, R. Willeke and R. Tacke, *Organosilcon Chem.* **IV**, 2000, 33.
391. M. Mohamed and M. A. Brook, *Tetrahedron Lett*., **42**, 2001, 191.
392. T. Kawamoto, H. Yamamnaka and A. Tanaka, *Appl. Biochem. Biotechnol*., **88**, 2000, 17.
393. M. Pietzsch, T. Waniek, R. J. Smith, S. Bratovanov, S. Bienz and C. Syldatk, *Monatsh. Chem*., **131**, 2000, 645.
394. C. Bolm, A. Kasyan, K. Drauz, K. Gunter and G. Raabe, *Angew. Chem., Int. Ed*., **39**, 2000, 2288.
395. B. Vivet, F. Cavelier, J. Martinez, *Eur. J. Org. Chem*., **5**, 2000, 807.
396. E. Alonso, E. Alonso, A. Solis and C. Del Pozo, *Synlett*., 2000, 698.
397. A. Wiemann, R. Frank and W. Tegge, *Tetrahedron*, **56**, 2000, 1331.
398. F. Hammerschmidt, W. Lindner, F. Wuggenig and E. Zarbl, *Tetrahedron*: *Asymmetry*, **11**, 2000, 2955.
399. H. Chen, L. Bischoff, M.-C. Fournie-Zaluski and B. P. Roques, *J. Labelled Compd. Radiopharm*., **43**, 2000, 103.
400. S. Chetyrkina, K. Estieu-Gionnet, G. Lain, M. Bayle and G. Deleris, *Tetrahedron Lett*., **41**, 2000, 1923.
401. R. El Khalabi, A. El Halaoui, F. Ouazzani, A. Elachqar, A. Atmani, M. L. Roumestant, P. Viallefont and J. Martinez, *Prep. Biochem. Biotechnol*., **30**, 2000, 295.
402. D. Ma and W. Zhu, *J. Org. Chem*., **66**, 2001, 348.
403. L. A. P. Silks, *Phosphorus, Sulfur Silicon Relat. Elem*., **136-138**, 1998, 611.
404. J. M. Sanderson, P. Singh, C. W. G. Fishwick and J. B. C. Findlay, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3227.
405. M. Abdaoui, G. Dewynter, L. Toupet and J.-L. Montero, *Tetrahedron*, **56**, 2000, 2427.
406. Z. S. Zhou, A. E. Smith and R. G. Mathews, *Bioorg. Med. Chem. Lett*., **10**, 2000, 2471.
407. D. G. I. Petra, P. C. J. Kamer, A. L. Spek, H. E. Schoemaker and P. W. N. M. Van Leeuwen, *J. Org. Chem*., **65**, 2000, 3010.
408. J. Poncet and P. Jouin, *Trends Org. Chem*., **7**, 1998, 123.
409. R. P. Schaum, *Diss. Abstr. Int*., **60**, 2000, 5097.
410. R. Ponsinet, G. Chassaing, J. Vaissermann and S. Lavielle, *Eur. J. Org. Chem*., **1**, 2000, 199.
411. V. P. Kukhar, *Aminophosphonic Aminophosphinic Acids*, 2000, 75.
412. H. Miyabe, K. Fujii, M. Ueda and T. Naito, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, 2000, 42nd, 601, *Chem. Abs*., 2001, 101979.
413. S. G. Nelson and K. L. Spencer, *Angew. Chem. Int. Ed*., **39**, 2000, 1323.
414. V. Mickevicius and B. Sapiyanskaite, *Chem. Heterocycl. Compd.* (*NY*), **35**, 2000, 1433.

415. Z. J. Beresnevicius, V. Viliunas and K. Kantminiene, *Chem. Hetercycl. Compd.* (*N Y*), **36**, 2000, 432.
416. H. Ishitani, M. Ueno and S. Kobayashi, *J. Am. Chem. Soc*., **122**, 2000, 8180.
417. P. Allef and H. Kunz, *Tetrahedron*: *Asymmetry*, **11**, 2000, 375.
418. T. Kawakami, H. Ohtake, H. Arakawa, T. Okachi, Y. Imada and S.-I. Murahashi, *Bull. Chem. Soc. Jpn*., **73**, 2000, 2423.
419. G. Nagula, V. J. Huber, C. Lum and B. A. Goodman, *Org. Lett*., **2**, 2000, 3527.
420. A. B. McLaren and J. B. Sweeney, *Synlett*, 2000, 1625.
421. S. D. Bull, S. G. Davies, S. Delgado-Ballester, G. Fenton, P. M. Kelly and A. D. Smith, *Synlett*, 2000, 1257.
422. C. Agami, S. Cheramy, L. Dechoux and M. Melaimi, *Tetrahedron*, **57**, 2001, 195.
423. N. Kise and N. Ueda, *Org. Lett*., **1**, 1999, 190.
424. H.-J. Gais, R. Loo, P. Das and G. Raabe, *Tetrahedron Lett*., **41**, 2000, 2851.
425. J. R. Casimir, C. Didierjean, A. Aubry, M. Rodriguez, J.-P. Briand and G. Guichard, *Org. Lett*., **2**, 2000, 895.
426. M. Calmes, F. Escale, E. Juan, M. Rolland and J. Martinez, *Tetrahedron*: *Asymmetry*, **11**, 2000, 1263.
427. F. Benedetti, P. Maman and S. Norbedo, *Tetrahedron Lett*., **41**, 2000, 10075.
428. M. Brenner and D.Seebach, *Helv. Chim. Acta*, **82**, 1999, 2365.
429. M. Catasus, A. Moyana, M. A. Pericas and A. Riera, *Tetrahedron Lett*., **40**, 1999, 9309.
430. P. O'Brien, D. W. Porter and N. M. Smith, *Synlett*, 2000, 1336.
431. M. Martin-Vila, E. Muray, G. P. Aguada, A. Alvarez-Larena , V. Branchadell, C. Minguillon, E. Giralt and R. M. Ortuna, *Tetrahedron*: *Asymmetry*, **11**, 2000, 3569.
432. R. Beumer, C. Bubert, C. Cabrele, O. Vielhauer, M. Pietzsch and O. Reiser, *J. Org. Chem*., **65**, 2000, 8960.
433. F. Fulup, *Farmaco*, **55**, 2000, 181.
434. D. A. Price, *Synlett*, **2**, 1999, 1919.
435. M. Kordes, H. Winsel and A. De Meijere, *Eur. J. Org. Chem*., 2000, 3250.
436. F. A. Davis, T. Fang, B. Chao and D. M. Burns, *Synthesis*, 2000, 2106.
437. G. D. Monache, D. Misiti and G. Zappia, *Tetrahedron*: *Asymmetry*, **10**, 1999, 2961.
438. M. Bendl, M. Eder, I. Langhammer and E. Urban, *Heterocycles*, **53**, 2000, 115.
439. J. S. Norwick, D. M. Chung, K. Maitra, S. Maitra, K. D. Stigers and Y. Sun, *J. Amer. Chem. Soc*., **122**, 2000, 7654.
440. D. Zicane, I. Ravinya, I. Rijkure, E. Gudrinieec and U. Kalejs, *Chem. Heterocycl. Compd.* (*N Y*), **36**, 2000, 754.
441. R. Pascal, R. Sola, F. Labeguere and P. Jouin, *Eur. J. Org. Chem*., 2000, 3755.
442. S. Okamoto, K. Kukuhara and F. Sato, *Tetrahedron Lett*., **41**, 2000, 5561.
443. K. Ananda, H. N. Gopi and V. V. S. Babu, *J. Pept. Res*., **55**, 2000, 289.
444. Y.-Y. Hong, B.-R. Liao, B. Liu and F. Xiong, *Youji Huaxue*, **20**, 2000, 367; *Chem. Abs*., 2000, 363743.
445. P. M. T. Ferreira, H. L. S. Maia, L. S. Monteiro, J. Sacramento and J. Sebastiao, *J. Chem. Soc*., *Perkin Trans. 1*, 2000, 3317.
446. S. G. Davies and O. Ichihara, *Tetrahedron Lett*., **40**, 1999, 9313.
447. G. Guanti, A. Moro and E. Narisano, *Tetrahedron Lett*., **41**, 2000, 3203.
448. H. Yang, K. Foster, C. R. J. Stephenson, W. Brown and E. Roberts, *Org. Lett*., **2**, 2000, 2197.
449. H. N. Gopi and V. V. S. Babu, *Lett. Pept. Sci*., **7**, 2000, 165.
450. J. L. Garcia Ruano and C. Garcia Paredes, *Tetrahedron Lett*., **41**, 2000, 5357.

451. S.-H. Shin, E. H. Han, C. S. Park, W. K. Lee and H.-J. Ha, *Tetrahedron: Asmmetry*, **11**, 2000, 3293.
452. R. E. Dolle, T. F. Herpin and Y. C. Shimshock, *Tetrahedron Lett*., **42**, 2001, 1855.
453. T. Sonke, B. Kaptein, W. H. J. Boesten, Q. B. Broxterman, H. E. Shoemaker, J. Kamphuis, F. Formaggio, C. Toniolo and F .P. J. T. Floris, *Stereosel. Biocatal*., 2000, 23.
454. V. Schurig, *J. Chromatogr*., **906**, 2001, 275.
455. X.-Y. Pang, M. Ya and Z.-Y. Li, *Yingyong Huaxue*, **17**, 2000, 105.
456. T. Shiraiwa, M. Kubo, K. Fukuda and H. Kurokawa, *Biosci. Biotechnol. Biochem*., **63**, 1999, 2212.
457. V. Tolman and P. Simek, *J. Fluorine Chem*., **101**, 2000, 11.
458. V. M. Profir and M. Matsuoka, *Colloids Surf*., **164**, 2000, 15.
459. R. Yoshioka, H. Hiramatsu, K. Okamura, I. Tsujioka and S.-I. Yamada, *J. Chem. Soc*., *Perkin Trans. 2*, 2000, 2121.
460. S. J. Faulconbridge, K. E. Holt, L. G. Sevillano, C. J. Lock, P. D. Tiffin, N. Tremayne and S. Winter, *Tetrahedron Lett*., **41**, 2000, 2679.
461. T. Miyazawa, H. Iwanaga, S. Ueji and T. Yamada, *Biocatal. Biotransform*., **17**, 2000, 445.
462. Y. Abe, T. Shoji, M. Matsubara, M. Yoshida, S. Sugata, K. Iwata and H. Suzuki, *Chirality*, **12**, 2000, 565.
463. Y. N. Belokon, K. A. Kochetkov, F. M. Plieva, N. S. Ikonnikov, V. I. Maleev, V. S. Parmar, R. Kumar and V. I. Lozinsky, *Appl. Biochem. Biotechnol*., **88**, 2000, 97.
464. K. Tomisaka, Y. Ishida, K. Konishi and T. Aida, *Chem. Commun*., **1**, 2002, 233.
465. D. J. Bennett, K. I. Buchanan, A. Cooke, O. Epemolu, N. M. Hamilton, E. J. Hutchinson and A. Mitchell, *J. Chem. Soc*., *Perkin Trans. 1*, 2001, 362.
466. S. A. Brow, M.-C. Parker and N. J. Turner, *Tetrahedron: Asymmetry*, **11**, 2000, 1687.
467. J. D. Scott and R. M. Williams, *Tetrahedron Lett*., **41**, 2000, 8413.
468. A. Basso, P. Braiuca, L. De Martin, C. Ebert, L. Gardossi and P. Linda, *Tetrahedron: Asymmetry*, **11**, 2000, 1789.
469. V. Tolman and P. Simek, *J. Fluorine Chem*., **101**, 2000, 5.
470. T. Furutani and M. Furui, *Biotechnol Lett*., **21**, 1999, 1101.
471. R. Bhushnan, J. Martens and G. T. Thiongo, *J. Pharm. Biomed Anal*., **21**, 2000, 1143.
472. R. Bhushnan and G. T. Thiongo, *J. Planar Chromatog.-Mod. TLC*, **13**, 2000, 33.
473. J. W. LeFevre, E. D. Rogers, L. L. Pico and C. L. Botting, *Chromatographia*, **52**, 2000, 648.
474. M. H. Hyun, Y. D. Kim and S. C. Han, *J. High Resolut. Chromatogr*., **23**, 2000, 333.
475. A. Peter, E. Olajos, R. Casimir, D. Tourwe, Q. B. Broxterman, B. Kaptein and D. W. Armstrong, *J. Chromatogr*., **871**, 2000, 105.
476. A. Peter, M. Peter, F. Fulop, G. Turok, G. Toth, D. Tourwe and J. Sapi, *Chromatographia*, **51 (Suppl.)**, 2000, S148.
477. G. Torok, A. Peter, E. Vekes, J. Sapi, M. Laronze, J.-Y. Laronze and D. W. Armstrong, *Chromatographia*, **51 (Suppl.)**, 2000, S165.
478. K. Chen, Y.-Q. Chen, X.-R. Xu, Y.-J. Yu, Z.-Q. Guo and X.-M. Jiang, *Sepu*, **18**, 2000, 14; *Chem. Abs*., **132**, 27368.
479. G. Grigorean, J. Ramirez, S. H. Ahn and C. B. Lebrilla, *Anal. Chem*., **72**, 2000, 4275.
480. W. A. Tao, D. Zhang, E. N. Nikolaev and R. G. Cooks, *J. Am. Chem. Soc*., **122**, 2000, 10598.
481. M. Lodder, B. Wang and S. M. Hecht, *Tetrahedron*, **56**, 2000, 9421.
482. M. Schlauch and A. W. Frahm, *Anal. Chem*., **73**, 2001, 262.

483. S. Zhao and Y.-M. Liu, *Anal. Chem. Acta*, **426**, 2001, 65.
484. O. Vandenabeele-Trambouze, M. Albert, C. Bayle, F. Couderc, A. Commeyras, D. Despois, M. Dobrijevic and M.-F. Grenier Loustalot, *J. Chromatogr., A*, **894**, 2000, 259.
485. Z. Q. Ruan, J.-B. Li, H.-J. Lu and Q.-Y.Ou, *Sepu*, **18**, 2000,148; *Chem. Abs.*, 2000, 287851.
486. G. Thorsen and J. Bergquist, *J. Chromatogr., B: Biomed. Sci. Appl.*, **745**, 2000, 389.
487. D. Kaniansky, E. Simunicova and E. Olvecka, *Electrophoresis*, **20**, 1999, 2786.
488. S. Szabo, R. Khlafulla, S. Szarvas, L. Ladanyi and G. Szokan, *Chromatagraphia*, **51 (Suppl.)**, 2000, 193.
489. I.-J. Kim, S.-J. Park and H.-J. Kim, *J. Chromatogr., A*, **877**, 2000, 217.
490. S. Ahn, J. Ramirez, G. Grigorean and C. B. Lebrilla, *J. Amer. Soc. Mass Sprectrom.*, **12**, 2001, 278.
491. B. Kaptein, H. Elsenberg, R. F. P. Grimbergen, Q. B. Broxterman, L. A. Hulshof, K. L. Pouwer and T. Vries, *Tetrahedron: Asymmetry*, **11**, 2000, 1343.
492. M. Calmes, C. Glot, T. Michel, M. Rolland and J. Martinez, *Tetrahedron: Asymmetry*, **11**, 2000, 737.
493. S. Allenmark and B. Lamm, *Chirality*, **13**, 2001, 43.
494. M. Yoshida, K. Ueza, M. Goto and S. Furasaki, *J. Appl. Polym. Sci.*, **78**, 2000, 695.
495. Y.-D. Long, T.-B. Huang, *Hecheng Huaxue*, **8**, 2000, 317; *Chem. Abs.*, **133**, 316988.
496. P. E. M.Overdevest, A. van der Padt, J. T. F. Keurentjes and K. van't Riet, *Colloids Surf.*, **163**, 2000, 209.
497. T. J. M. De Bruin, A. T. M. Marcelis, H. Zuilhof, L. M. Rodenburg, H. A. G. Niederlander, A. Koudijs, P. E. M. Overdevest, A. Van Der Padt and E. J. R. Sudholter, *Chirality*, **12**, 2000, 627.
498. G. Lu, F. Liu, W. Song, W. He, P. Prados and J. De Mendoza, *Mater. Sci. Eng.*, **C10**, 1999, 29.
499. S. Yagi, H. Sadachi, Y. Kashiwagi, T. Takagishi, T. Mizutani, S. Kitagawa and H. Ogoshi, *Chem. Lett.*, 2000, 1054.
500. P. Luger and M. Weber, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, **C56**, 2000, 1882.
501. B. Dalhus and C. H. Gorbitz, *Acta Crystallog., Sect. B: Struct. Sci.*, **B56**, 2000, 720.
502. W. J. Liu, C. Ferrari, M. Zha, L. Zanotti and S. S. Jiang, *Cryst. Res. Technol.*, **35**, 2000, 1215.
503. A. S. Pandi, S. Muthu, S. Gunasekaran and M. Dakshinamoorthy, *Asian J. Phys.*, **9**, 2000, 505.
504. R. Kingsford-Adaboh, M. Grosche, B. Dittrich and P. Luger, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, **C56**, 2000, 1274.
505. Y.-X. Che, J.-M. Zheng, J.-M. Hao and L.-Q. Chu, *Fenxi Ceshi Xuebao*, **19**, 2000, 45; *Chem. Abs.*, **134**, 155437.
506. R. Puliti and C. A. Mattia, *Acta Crystallog., Sect. C: Cryst. Struct. Commun.*, **C56**, 2000, 496.
507. K. L. Stout, K. J. Hallock, J. W. Kampf and A. Ramamoorthy, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, **C56**, 2000, 100.
508. V. H. Rodrigues, J. A. Paixao, M. M. R. R. Costa and A. M. Beja, *Acta Crystallog., Sect. C: Cryst. Struct. Commun.*, **C56**, 2000, 1053.
509. D. Madsen, P. Pattison, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, **C56**, 2000, 1157.
510. A. B. Hughes, M. F. MacKay and L. Aurelio, *Aust. J. Chem.*, **53**, 2000, 237.
511. H. Ratajczak, J. Barycki, A. Pietraszko, J. Baran, S. Debrus, M. May and J.

Venturini, *J. Mol. Struct*., **526**, 2000, 269.
512. M. J. Potrzebowski, P. Tekely, J. Blaszczyk and M. W. Wieczorek, *J. Pept. Res*., **56**, 2000, 185.
513. Y. Gong, K. Kato and H. Kimoto, *Anal. Sci*., **16**, 2000, 893.
514. J. Zhuang, X. Zheng, F. Li, X. Li, L. Jin and J. Guo, *J. Mol. Struct*., **522**, 2000, 255.
515. J. V. Pratap, R. Ravishankar and M. Vijayan, *Acta Crystallog*., *Sect. B*: *Struct. Sci*., **B56**, 2000, 690.
516. D. R. van Staveren, T. Weyheymueller, and N. Metzler-Nolte, *Organometallics*, **19**, 2000, 3730.
517. J. N. Low, M. D. Lopez, P. A. Mascaros, J. C. Domingo, M. L. Godino, R. L. Garzon, M. D. Gutierrez, M. Melguizo, G. Ferguson and C. Glidewell, *Acta Crystallogr*., *Sect. B*: *Struct. Sci*., **B56**, 2000, 882.
518. X. Zheng, I. O. Donkor, D. D. Miller and C. R. Ross, *Chirality*, **12**, 2000, 2.
519. X. Yan, W. Zhu, S. Zhu, H. Lin, Z. Zhu and R. Chen, *Beijing Shifan Daxue Xuebao*, *Ziran Kexueban*, **36**, 2000, 221; *Chem. Abs*., **133**, 208136.
520. L. G. R. Hammarstrom, T. Zhang, J. Giraldes, M. L. McLaughlin, D. R. Billodeaux and F R. Fronczek, *Acta Crystallogr*., *Sect. C*: *Cryst. Struct. Commun*., **C56**, 2000, 1484.
521. E. Bunuel, C. Cativiela, M. D. Diaz-De-Villegas and J. A. Galvez, *Acta Crystallogr*., *Sect. C*: *Cryst Commun*., **C56**, 2000, 587.
522. T. Kassem, V. Rolland, J. Martinez and M. Rolland, *Acta Crystallogr*., *Sect. C*: *Cryst. Struct. Commun*., **C56**, 2000, 1037.
523. Y. In, M. Fujii, Y. Sasada and T. Ishida, *Acta Crystallogr*., *Sect. B*: *Struct. Sci*., **B57**, 2001, 72.
524. P. A. Nielsen, J. W. Jaroszewski, P.-O. Norrby and T. Liljefors, *J. Am. Chem. Soc*., **123**, 2001, 2003.
525. A. Ozarowski and A. H. Maki, *J. Phys. Chem*., **104**, 2000, 1022.
526. P. Chalard, M. Bertrand, I. Canet, V. Thery, R. Remuson and G. Jeminet, *Org. Lett*., **2**, 2000, 2431.
527. R. J. Abraham, N. Aboitiz, J. Merrett, B. Sherbourne and I. Whitcombe, *J. Chem. Soc*., *Perkin Trans. 2*, 2000, 2382.
528. K. Ganicz, *Bull. Pol. Acad. Sci*, *Chem*., **48**, 2000, 57.
529. M. A. Earle and P. G. Hultin, *Tetrahedron Lett*., **41**, 2000, 7855.
530. A. V. Vashchuk, M. A. Marusov, O. A. Pilyuk, V. A. Volynkin and V. T. Panyushkin, *Russ. J. Gen. Chem*., **69**, 1999, 1628.
531. D. Jedrzejczyk, Z. Ziora and P. Kafarski, *Magn. Reson. Chem*., **38**, 2000, 370.
532. T. J. Wenzel and J. E. Thurston, *Tetrahedron Lett*., **41**, 2000, 3769;
533. T. J. Wenzel and J. E. Thurston, *J. Org. Chem*., **65**, 2000, 1243.
534. A. Inamoto, K. Ogasawara, K. Omata, K. Kabuto and Y. Sasaki, *Org. Lett*., **2**, 2000, 3543.
535. Z. Li, L. Zhou, Z. Xia, Y. Liu, M. Zhang, H. Peng, S. Liu and B. Yu, *Bopuxue Zazhi*, **17**, 2000, 309, *Chem. Abs*., **133**, 296040.
536. V. Tsikaris, V. Moussis, M. Sakarellos-Daitsiotis and C. Saarellos, *Tetrahedron Lett*., **41**, 2000, 8651.
537. A. M. Petrosan, V. M. Burbelo, R. A. Tamazyan, H. A. Karapetyan and R. P. Sukiasyan, *Z. Naturforsch*., *A*: *Phys. Sci*., **55**, 2000, 199.
538. L. F. Krylova and A. V. Golovin, *J. Struct. Chem*., **41**, 2000, 243.
539. L. F. Krylova and A. V. Golovin, *J. Struct. Chem*., **41**, 2000, 254.
540. J. R. Sachleben, P. Beverwyk and L. Frydman, *J. Magn. Reson*., **144**, 2000, 330.
541. V. T. Panyushkin, and V. A. Volykin, *J. Struct. Chem*., **41**, 2000, 541.

542. S. G. Allenmark, *Nat. Prod. Rep.*, **17**, 2000, 145.
543. P. Skowronek and J. Gawronski, *Tetrahedron: Asymmetry*, **10**, 1999, 4585.
544. I. Powis, *J. Phys. Chem.*, **104**, 2000, 878.
545. G. R. Asbury and H. H. Hill, *J. Chromatogr.*, **902**, 2000, 433.
546. F. Rogalewicz, Y. Hoppilliard and G. Ohanessian, *Int. J. Mass Spectrom.*, **195**/**196**, 2000, 565.
547. Y. Ji, X. Li and S. Liu, *Fujian Fenxi Ceshi*, **8**, 1999, 1093.
548. D. Zhang, L. Wu, K. J. Koch and R. G. Cooks, *Eur. J. Mass Spectrom.*, **5**, 1999, 353.
549. F. Piuzzi, I. Dimicoli, M. Mons, B. Tardivel and Q. Zhao, *Chem. Phys. Lett.*, **320**, 2000, 282.
550. Z.-P. Yao, T. S. M. Wan, K.-P. Kwong and C.-T. Che, *Anal. Chem.*, **72**, 2000, 5383.
551. Z.-P. Yao, T. S. M. Wan, K.-P. Kwong and C.-T. Che, *Anal. Chem.*, **72**, 2000, 5394.
552. M. P. So, T. S. M. Wan and T. W. D. Chan, *Rapid Commun. Mass Spectrom.*, **14**, 2000, 692.
553. F. Rogalewicz, Y. Hoppilliard and G. Ohanessian, *Int. J. Mass Spectrom.*, **206**, 2001, 45.
554. F. Rogalewicz, Y. Hoppilliard and G. Ohanessian, *Int. J. Mass Spectrom.*, **204**, 2001, 267.
555. M. Kaussmann and H. Budzikiewicz, *Spectroscopy* (*Amsterdam*), **14**, 1999, 67.
556. C. Bruyneel, A. K. Chandra, T. Uchimara and T. Zeegers-Huyskens, *Spectrochim. Acta*, *Part A*, **56**, 2000, 591.
557. B. J. M. Rajkumar and V. Ramakrishnan, *J. Raman Spectrosc.*, **31**, 2000, 1107.
558. X. Cao and G. Fischer, *J. Mol. Struct.*, **519**, 2000, 153.
559. J. R. Carney and T. S. Zwier, *J. Phys. Chem.*, **104**, 2000, 8677.
560. L. C. Snoek, E. G. Robertson, R. T. Kroemer and J. P. Simons, *Chem. Phys. Lett.*, **321**, 2000, 49.
561. M. Sonoyama, H. Yajima, T. Nakano, M. Matsumoto, I. Tsukushi and T. Ishii, *Chem. Lett.*, 2000, 552.
562. X. Cao and G. Fischer, *Chem. Phys.*, **255**, 2000 195.
563. K. Frimand, H. Bohr, K. J. Jalkanen and S. Suhai, *Chem. Phys.*, **255**, 2000, 165.
564. R. P. Varma and J. Parshad, *Tenside*, *Surfactants*, *Deterg.*, **36**, 1999, 330.
565. X. Zhang, W. Li, H. Jia and S. Weng, *Guangpuxue Yu Guangpu Fenxi*, **20**, 2000, 185, *Chem. Abs.*, 2000, 318275.
566. H.-W. Li and H. L. Strauss, *J. Phys. Chem.*, *B*, **105**, 2001, 2250.
567. E. J. Baran, C. C. Wagner, M. H. Torre, E. Kremer and P. Kogerler, *Acta Farm. Bonaerense*, **19**, 2000, 231.
568. B. L. Silva, P. T. C. Freire, F. E. A. Melo, J. M. Filho, M. A. Pimenta and M. S. S. Dantas, *J. Raman Spectrosc.*, **31**, 2000, 519.
569. A. M. R. Teixeira, P. T. C. Freire, A. J. D. Moreno, J. M. Sasaki, A. P. Ayala, J. Mendes Filho and F. E. A. Melo, *Solid State Commun.*, **116**, 2000, 405.
570. C. Bruyneel and T. Huyskens, *J. Mol. Struct.*, **552**, 2000, 177.
571. D. Zhang, S. Sun, Z. Ma, G. Chen and F. Cui, *Hejishu*, **23**, 2000, 213, *Chem. Abs.* 2000, 554779.
572. J. Han, Z. Yu, *Guangpuxue Yu Guangpu Fenxi*, **19**, 1999, 779.
573. V. S. Gorelik, A. V. Chervyakov, L. I. Zlobina and O. N. Sharts, *Pro. SPIE-Int. Soc. Opt. Eng.*, 3855 (*Air Monitoring and Detection of Chemical and Biological Agents*, *II*), 1999, 16; *Chem. Abs.*, **132**, 278866.
574. H. M. Saleh and S. M. Al-Ghannam, *Alexandria J. Pharm. Sci.*, **14**, 2000, 25.
575. M. A. Dempster and R. K. Marcus, *J. Anal. At. Spectrom.*, **15**, 2000, 43.
576. B. Geisser, T. Skrivanek, U. Zimmermann, D. J. Stufkens and R. Alsfasser, *Eur. J.*

Inorg. Chem., 2001, 439.
577. I. J. Pickering, G. N. Graham, V. Van Fleet-Stalder, T. G. Chasteen and R. C. Prince, *J. Biol. Inorg. Chem.*, **4**, 1999, 791.
578. S. Cakir, E. Bicer and O. Cakir, *Glycoconjugate J.*, **16**, 2000, 579.
579. T. Mizutani, K. Wada, and S. Kitagawa, *J. Org. Chem.*, **65**, 2000, 6097.
580. J. Wang, Z. Yan, K. Zhuo and D. Lui, *Z. Phys. Chem.*, (*Muenchen*), **214**, 2000, 333.
581. C. M. Romero and J. L. Rojas, *Phys. Chem. Liq.*, **38**, 2000, 237.
582. Z. Yan, J. Wang, J. Lu, *J. Chem. Eng. Data*, **46**, 2001, 217.
583. J.-L. Shen, Z.-F. Li, B.-H. Wang and Y.-M. Zhang, *J. Chem. Thermodyn.*, **32**, 2000, 805.
584. Q. Liu, X. Hu, R. Lin, W. Sang and S. Li, *J. Chem. Eng. Data*, **46**, 2001, 522.
585. R. G. Clarke, P. Tremaine, L. Hnedkovsky and V. Majer, *Steam, Water, Hydrotherm. Syst.: Proc. Int. Conf. Prop. Water Steam*, 13*th*, 2000, 221, *Chem. Abs.*, 2000, 682013.
586. R. G. Clarke, L. Hnedkovsky, P. Tremaine and V. Majer, *J. Phys. Chem. B*, **104**, 2000, 11781.
587. S. L. Gao, Y. D. Hou, M. Ji, S. P. Chen and Q. Z. Shi, *Thermochem. Acta*, **35**, 2000, 47;
588. B. Palecz, *Fluid Phase Equil.*, **167**, 2000, 253.
589. B. Palecz, H. Piekarski and S. Romanowski, *J. Mol. Liq.*, **84**, 2000, 279.
590. X. Ren, Y. Ni and R. Lin, *Thermochim. Acta*, **348**, 2000, 19.
591. S. Shao, X. Hu and R. Lin, *Thermochim. Acta*, **360**, 2000, 93.
592. S. Li, X. Hu, R. Lin and H. Zong, *Thermochim. Acta*, **342**, 1999, 1.
593. X. Ren, Y. Ni and R. Lin, *Wuli, Huaxue Xuebao*, **16**, 2000, 166; *Chem. Abs.* 2000, 165152.
594. X. Wei, X. Hu, S. Shao, R. Lin and S. Li, *Thermochim. Acta*, **362**, 2000, 1.
595. H. Gao, X. Hu and R. Lin, *Thermochim. Acta*, **346**, 2000, 1.
596. M. Xian, X.-Q. Zhu, J. Lu, Z. Wen and J.-P. Cheng, *Org. Lett.*, **2**, 2000, 265.
597. S. Shao, X.-G. Hu, and R.-S. Lin, *Huaxue Xuebao*, **58**, 2000, 1240; *Chem. Abs.*, 2000, 792990.
598. L. Gang, R. Lin and H. Zong, *Huaxue Xuebao*, **16**, 2000, 188; *Chem. Abs.*, 2000, 165176.
599. J. Wang, Z. Yan, H. Zhang and J. Lu, *Biophys. Chem.*, **86**, 2000 71.
600. A. W. Hakin, J. L. Liu and R. A. Marriott, *J. Chem. Thermodyn.*, **32**, 2000, 1365.
601. G. C. Bag, N. M. Singh and N. J. Singh, *J. Indian Chem. Soc.*, **77**, 2000, 146.
602. I. V. Aristov, O. V. Bobreshova, S. Y. Eliseev and P. I. Kulintsov, *Russ. J. Electrochem.*, **36**, 2000, 323.
603. G. C. Bag, N. R. Singh and N. R. Singh, *Chem. Environ. Res.*, **7**, 1998, 221.
604. K. Majumder, K. Majumder and S. C. Lahiri, *Z. Phys. Chem.*, (*Muenchen*), **214**, 2000, 285.
605. Z. Sarkelov, C. I. Dzhumalieva, T. A. Toktomatov and K. R. Rysmendeev, *Izv. Nats. Akad. Kyrg. Resp.*, 2000, 24; *Chem. Abs.*, **134**, 168866.
606. Y. Kim, *J. Mater. Sci.*, **35**, 2000, 873.
607. L. V. Kuritsyn, N. V. Kalinina and L. N. Khripkova, *Zh. Fiz. Khim.*, **74**, 2000, 1721; *Chem. Abs.*, **134**, 42400.
608. E. Bottari, D. Cellulosi, M. R. Festa, *Talanta*, **50**, 1999, 993.
609. P. Ma, Y. Wu, Y. Liu, S. Xia and S. Li, *Chin. J. Chem. Eng.*, **8**, 2000, 146.
610. S. Koutsopoulos and E. Dalas, *Langmuir*, **16**, 2000, 6739.
611. S. Koutsopoulos and E. Dalas, *Langmuir*, **17**, 2001, 1074.
612. T. Kijima, K. Yamaguchi, A. Miyata, M. Yada and J. Tanaka, *Chem. Lett.*, 2000,

1324.
613. A. Kai and T. Miki, *J. Appl. Phys., Part* 2, **39**, 2000, L1071.
614. M. Yokota, M. Mochizuki, K. Saito, A. Sato and N. Kubota, *Chem. Eng. Commun.*, **174**, 1999, 243.
615. A. Li, C. Xu, A. Li and N. Ming, *J. Crystal Growth*, **220**, 2000, 291.
616. J. Yano, H. Fueredi-Milhofer, E. Wachtel and N. Garti, *Langmuir*, **16**, 2000, 9996.
617. A. A. Pradhan and J. H. Vera, *J. Chem. Eng. Data*, **45**, 2000, 140.
618. S.-L. Gao, Y.-D. Hou, J.-R. Liu and S.-P. Chen, *Huaxue Xuebao*, **58**, 2000, 65; *Chem. Abs.*, **132**, 194629.
619. Y.-F. Hu, Y.-H. Liu and S.-P. Sun, *J. Chem. Thermodn.*, **32**, 2000, 249.
620. T. Imae, N. Hayashi, T. Matsumoto, T. Tada and M. Furusaka, *J. Colloid Interface Sci.*, **225**, 2000, 285.
621. F. M. Menger and K. L. Caran, *J. Am. Chem. Soc.*, **122**, 2000, 11679.
622. L. Rodehuser, H. Chaumette, A. Meyers, E. Rogalska, C. Gerardin and C. Selve, *Amino Acids*, **18**, 2000, 89.
623. A. Kabir-ud-Din, J. K. J. Salem, S. Kumar and Z. Khan, *Colloids, Surf., A*, **168**, 2000, 241.
624. L. Weng, S. Wang, X. Cai and M. Xiao, *Huaqiao Daxue Xuebao*, *Ziran Kexueban*, **21**, 2000, 189; *Chem. Abs.*, **133**, 26294.
625. Y.-S. Liu, Y.-Y. Dai and J.-D. Wang, *Sep. Sci. Technol.*, **35**, 2000, 1439.
626. M. Miyanaga, K. Inamura, K. Tanaka, T. Sakiyama and K. Nakanishi, *J. Biosci. Bioeng.*, **88**, 1999, 651.
627. N. Okuyama-Yoshida, K. Kataoka, M. Nagaoka and T. Yamabe, *J. Chem. Phys.*, **113**, 2000, 3519.
628. I. Tunon, E. Silla and M. F. Ruiz-Lopez, *Chem. Phys. Lett.*, **32**, 2000, 433.
629. B. Noszal, D. Visky and M. Kraszni, *J. Med., Chem.*, **43**, 2000, 2176.
630. P. I. Nagy and B. Noszal, *J. Phys. Chem.*, **104**, 2000, 6834.
631. M. Ramek and P. I. Nagy, *J. Phys. Chem.*, **104**, 2000, 6844.
632. X. Zhang, Y. Liu, J. Sun and Q. Liu, *Wuli*, *Huaxue Xuebao*, **16**, 2000, 351; *Chem. Abs.*, 2000, 294132.
633. F. Koseoglu, E. Kilic, A. Dogan, *Anal. Biochem.*, **277**, 2000, 243.,
634. M. S. Babu, G. N. Rao, K. V. Ramana and M. S. P. Rao, *J. Indian Chem. Soc.*, **77**, 2000, 380.
635. A. Fernandez-Ramos, Z. Smedarchina, W. Siebrand and M. Z. Zgierski, *J. Chem. Phys.*, **113** 2000, 9714.
636. F. Tortonda, E. Silla, I. Tunon, D. Rinaldi and M. F. Ruiz-Lopez, *Theor. Chem. Acc.*, **104**, 2000, 89.
637. S. J. Grabowski, T. M. Krygowski and B. Stepien, *J. Phys. Org. Chem.*, **13**, 2000, 740.
638. J. M. Antelo, F. Arce, P. Calvo, J. Crugeiras and A. Rios, *J. Chem. Soc., Perkin Trans. 2*, 2000, 2109.
639. R. E. D. McClung, T. T. Nakashima, H. Yamamoto and G. Kotovych, *Can. J. Chem.*, **77**, 1999, 1728.
640. A. Rios, T. L. Amyes and J. P. Richard, *J. Am. Chem. Soc.*, **122**, 2000, 9373.
641. T. Kiss and I. Lazar, *Aminophosphonic Aminophosphinic Acids*, 2000, 285.
642. Z. Chen, K. Uchiyama and T. Hobo, *Anal. Sci.*, **16**, 2000, 837.
643. T. J. M. de Bruin, A. T. M. Marcelis, H. Zuilhof and E. J. R. Sudholter, *Langmuir*, **16**, 2000, 8270.
644. C. Conato, A. Contino, G. Maccarrone, A. Magri, M. Remelli and G. Tabbi, *Thermochim. Acta*, **362**, 2000, 13.
645. B. Gergas, S. Nikolic, N. Paulic and N. Raos, *Croat. Chem. Acta*, **72**, 1999, 885.

646. G. Branica, N. Paulic, B. Gergas and D. Omanovic, *Chem. Speciation Bioavailability*, **11**, 1999, 125.
647. N. M. Guindy, Z. M. Abou-Gamra and M. F. Abdel-Messih, *Monatsh. Chem.*, **131**, 2000, 857.
648. P. Gili and P. A. Lorenzo-Luis, *Trends Inorg. Chem.*, **5**, 1998, 105.
649. X. Yu, E. Gao, Z. Shu and H. Chen, *Huaxue Gongye Yu Gongchen* (*Tianjin*), **16**, 1999, 249; *Chem. Abs.*, **132**, 242551.
650. M. F. Grigor'eva and N. M. Kadyrkulova, *Vestn, S.-Peterb., Univ., Ser.* 4: *Fiz. Khim*, 1999, 73.
651. S.-L. Gao, Y.-D. Hou, Q. Wei, J.-R. Liu and X.-W. Yang, *Xibei Daxue Xueban, Ziran Kexueban*, **29**, 1999, 517.
652. S.-L. Gao, Y.-D. Hou, Q. Wei, J.-R. Liu and X.-W. Yang, *Xibei Daxue Xueban, Ziran Kexueban*, **29**, 1999, 517.
653. V. A. Sharnin, *Russ. J. Gen. Chem.*, **69**, 1999, 1368.
654. S. Vajhallya and F. Khan, *J. Inst. Chem.* (*India*), **71**, 1999, 11.
655. A. Zaidi and F. Khan, *J. Indian Chem Soc.*, **77**, 2000, 88.
656. A. Zaidi and F. Khan, *Bull. Electrochem.*, **16**, 2000, 119.
657. N. Niklas, O. Walter and R. Alsfasser, *Eur. J. Inorg. Chem.*, 2000, 1723.
658. G. U. Rani, and D. D. Manwal, *J. Electrochem. Soc. India*, **49**, 2000 13, 15, 17.
659. R. M. Patel, H. C. Pandey, K. B. Pandeya and G. N. Mukerjee, *Indian J. Chem. Sect. A*: *Inorg., Bio-inorg., Phys., Theor., Anal. Chem.*, **38A**, 1999, 850.
660. N. M. Guindi, *Egypt. J. Chem.*, **43**, 2000, 253.
661. L. H. Abdel-Rahman, *Ann. Chem.* (*Rome*), **90**, 2000, 479.
662. M. Saladini, D. Iacopino and L. Menabue, *J. Inorg. Bichem.*, **78**, 2000, 355.
663. A. G. W. Hodson and R. K. Thind, *Polyhedron*, **19**, 2000, 791.
664. G. Ye, C.-X. Wang and S.-S. Qu, *Wuhan Univ. J. Nat. Sci.*, **5**, 2000, 479.
665. D. D. Manwal and G. U. Rani, *Natl. Acad. Sci. Lett.* (*India*), **22**,1999, 52.
666. H. B. Silber, N. Ghajari and V. Maraschin, *J. Alloys Compd.*, **303**, 2000, 112.
667. R. Gupta, P. C. Vyas, M. Arora and R. Bapna, *Bull. Electrochem.*, **16**, 2000, 110.
668. J. Koska, C. Mui and C. A. Haynes, *Chem. Eng. Sci.*, **56**, 2001, 29.
669. S. Cakir, E. Bicer and O. Cakir, *J. Inorg. Biochem.*, **77**, 1999, 249.
670. O. Likhodi and T. V. Chalikian, *J. Am. Chem. Soc.*, **122**, 2000, 7860.
671. K. Sugawara, Y. Kameda, T. Usuki, O. Uemura and T. Fukunaga, *Bull. Chem. Soc. Jpn.*, **73**, 2000, 1967.
672. T. V. Eliseeva and V. A. Shaposhnik, *Russ. J. Electrochem.*, **36**, 2000, 64.
673. I. Eichwurzel, H. Stiel, K. Teuchner, D. Leupold, H. Scheer, Y. Salomon and A. Scherz, *Photochem. Photobiol.*, **72**, 2000, 204.
674. Y. N. Bakanina, E. A. Zhukova, I. F. Abdullin, E. H. Turova and G. K. Budnikov, *Russ. J. Gen. Chem.*, **70**, 2000, 837.
675. J. Contineanu, *Rev. Chim.* (*Bucharest*), **50**, 1999, 739).
676. M. A. V. Ribeiro Da Silva, M. D. M. C. Ribeiro Da Silva and L. M. N. B. F. Santos, *J. Chem. Thermodyn.*, **32**, 2000, 1037.
677. V. V. Lemanov, *Ferroelectrics*, **238**, 2000, 775.
678. S. Spange, C. Achmidt and H. R. Kricheldorf, *Langmuir*, **17**, 2001, 865.
679. R. Destro, P. Roversi, M. Barzaghi and R. E. Marsh, *J. Phys. Chem.*, **104**, 2000, 1047.
680. W. Wang, F. Yi, Y. Ni, Z. Zhao, X. Jin and Y. Tang, *J. Biol. Phys.*, **26**, 2000, 51.
681. A. M. Teixeira, P. T. C. Freire, A. J. D. Morenio, J. M. Sasaki, A. P. Ayala, F. J. Mendes and F. E. A. Melo, *Los Alamos Natl. Lab. Prepr. Arch., Condens Matter*, 2000, 1; *Chem. Abs.*, **133**, 30454.

682. S. Mukerji and T. Kar, *Mater. Res. Bull.*, **35**, 2000, 711.
683. J. R. Su, C. F. Zhu, Z. G. Zhu, D. Xu and D. R. Yuan, *Mater. Res. Bull.*, **35**, 2000, 977.
684. J. R. Su, C. F. Zhu, Z. G. Zhu, D. Xu and D. R. Yuan, *J. Alloys Compd.*, **310**, 2000, 139.
685. D. R. Coghlan, C. J. Easton and E. R. T. Tiekink, *Aust. J. Chem.*, **53**, 2000, 551.
686. H. Wang, Z. Chen and F. Wang, *Gongye Weishengwu*, **30**, 2000, 21; *Chem. Abs.*, **133**, 131350.
687. L. Weng, S. Wang, X. Cai and M. Meitian, *Huaqiao Daxue Xuebao*, *Ziran Kexueban*, **21**, 2000, 187; *Chem. Abs.*, **133**, 26294.
688. F. Xun, L. Junling, M. Ying, Z. Li and W. Debao, *Colloids Surf., A*, **179**, 2000, 1.
689. T. Nishiki, K. Nakamura and D. Kato, *Biochem. Eng. J.*, **4**, 2000, 189.
690. S. Wu, Z. Yan and Z. Wu, *Mo Kexue Yu Jishu*, **20**, 2000, 18; *Chem. Abs.*, **134**, 117488.
691. M. Takahashi, Y. Hattori, Y. Kawabata, *Kiyo-Suzuka Kogyo Koto Senmon Gakko*, **33**, 2000, 89; *Chem. Abs.*, 2000, 206724.
692. P. Dzygiel and P. Wieczorek, *J. Membr. Sci.*, **172**, 2000, 223.
693. G. L. Tulasi and S. Kumar, *AIChE J.*, **45**,1999, 2534.
694. G.-Y. Lu, X.-B. Wan, C.-M. Jin and W. Song, *Huaxue Xuebao*, **58**, 2000, 890; *Chem. Abs.*, **133**, 237975.
695. S. V. Smirnova, I. I. Torocheshnikova and I. V. Pletnev, *Russ. Chem. Bull.*, **49**, 2000, 953.
696. A. R. Mustafina, A. Y. Zhiganshina, S. V. Ferorenko, E. K. Kazakova, V. V. Skripacheva and A. I. Konovalev, *Russ. J. Coord. Chem.*, **26**, 2000, 507.
697. O. N. Khokhlova, V. F. Selemenev and V. Y. Khokhlov, *Zh. Fiz. Khim.*, **73**, 1999, 1067.
698. H. Kanazawa, M. Akamatsu and T. Onami, *Fukushima Daigaku*, *Kyoikugakubu Ronshu*, *Rika Hokoku*, **63**, 1999, 21.
699. G. Wu, C. Wang, Q. Zhao, J. Liu and T. Li, *Sichuan Daxue Xuebao*, *Ziran Kexueban*, **37**, 2000, 568; *Chem. Abs.*, 2000, 676149.
700. B. Deore, Z. Chen and T. Nagaoka, *Anal. Chem.*, **72**, 2000, 3989.
701. T. A. Krysanova, D. L. Kotova, V. F. Selemenev and O. I. Rozhnova, *Zh. Fiz. Khim.*, **74**, 2000, 335.
702. M. A. Tsvetnov, N. P. Morgun and V. V. Khabalov, *Khim. Tekhnol. Vody*, **22**, 2000, 252.
703. Z.-G. Zhao, M.-Z. M. Jin and Y.-Q. Liu, *Gaodeng Xuexiao Huxue Xuebao*, **21**, 2000, 1904; *Chem. Abs.*, 2001, 133139.
704. S. Kalra, C. K. Pant and M. S. Mehta, *Indian J. Biochem. Biophys.*, **37**, 2000, 341.
705. J. Zhang, Q. Chi, J. U. Nielsen, E. P. Friis, J. E. T. Andersen and J. Ulstrup, *Langmuir*, **16**, 2000, 7229.
706. D. W. M. Arrigan and L. L. Bihan, *Analyst* (*Cambridge*, *UK*), **124**, 1999, 1645.
707. M. Nyberg, J. Hasselstrom, O. Karis, N. Wassdahl, M. Weinelt, A. Nilsson and L. G. M. Petterssen, *J. Chem. Phys.*, **112**, 2000, 5420.
708. H. Wang, X.-Y. Zhao and W.-S. Yang, *Wuli Xuebao*, **49**, 2000, 1316.
709. K. Imamura, T. Mimura, M. Okamoto, T. Sakiyama and K. Nakanishi, *J. Colloid Interface Sci.*, **229**, 2000, 237.
710. M. Sandow, B. L. May, P. Clements, C. J. Easton and S. F. Lincoln, *Aust. J. Chem.*, **52**, 1999, 1143.
711. C.-C. You and Y. Liu, *Gaodeng Xuexiao Huaxue Xuebao*, **21**, 2000, 249; *Chem. Abs.*, **132**, 280776.

712. G. Hembury, M. Rekharsky, A. Nakamura and Y. Inoue, *Org. Lett*., **2**, 2000, 3257.
713. Y. Liu, L. Jin and S.-X. Sun, *Microchem. J*., **64**, 2000, 59.
714. N. Van Hoof, and R. Darcy, *J. Inclusion Phenom. Macrocyclic Chem*., **36**, 2000, 179.
715. M. Rekharsky and Y. Inoue, *J. Am. Chem. Soc*., **122**, 2000, 10949.
716. O. I. Kalchenko, F. Perret, N. Morel-Desrosiers and A. W. Coleman, *J. Chem. Soc. Perkin Trans. 2*, 2001, 258.
717. M. Selkti, A. Tomas, A. W. Coleman, N. Douteau-Gueval, I. Nicolis, F. Villain and C. de Rango, *Chem. Commun*. (*Cambridge*), 2000, 161.
718. K. Ito and Y. Ohba, *Recent Res. Dev. Pure Appl. Chem*., **3**, 1999, 255.
719. K. Goto, Y. Murakami and R. Ueoka, *J. Mol. Catal. B*: *Enzym*., **11**, 2001, 985.
720. I. V. Terekhova and O. V. Kulikov, *Russ. Chem. Bull*., **48**, 1999, 2259.
721. H. J. Buschmann and L. Mutihac, *Ann. Chim*. (*Rome*), **90**, 2000, 369.
722. V. Jullian, E. Shepherd, T. Gelbrich, M. B. Hursthouse and J. D. Kilburn, *Tetrahedron Lett*., **41**, 2000, 3963.
723. M. Wehner, T. Schrader, P. Finocchiaro, S. Failla and G. Consiglio, *Org. Lett*., **2**, 2000, 605.
724. X.-b. Peng, J.-w. Huang, T. Li, L.-n. Ji, *Inorg. Chim. Acta*, **305**, 2000, 111.
725. M. Barboiu, C. Guizard, N. Hovnanian, J. Palmeri, C. Reibel, L. Cot and C. Luca, *J. Membr. Sci*., **172**, 2000, 91.
726. F. Garnier, J. Randon and J. L. Rocca, *Talanta*, **51**, 2000, 1001.
727. E. F. Strittmatter, A. S. Lemoff and E. R. Williams, *J. Phys. Chem. A*, **104**, 2000, 9793.
728. T. Wyttenbach, M. Witt and M. T. Bowers, *J. Am. Chem. Soc*., **122**, 2000, 3458.
729. M. J. Polce and C. Wesdemiotis, *J. Mass Spectrom*., **35**, 2000, 251.
730. R. Prabhakar, M. R. A. Blomberg and P. E. M. Siegbahn, *Theor. Chem. Acc*., **104**, 2000, 461.
731. Z. Li, H. Peng, Z. Xia, S. Liu, L. Zhou and B. Yu, *Bopuxue Zazhi*, **16**, 1999, 553; *Chem. Abs*. **132** 50234.
732. L. Sandberg and O. Edholm, *J. Phys. Chem*., *B*, **105**, 2001, 273.
733. M. Randic, D. Mills and S. C. Basak, *Int. J. Quantum Chem*., **80**, 2000, 1199.
734. F. Ban, J. W. Gould and R. J. Boyd, *J. Phys*., *Chem. A*, **104A**, 2000, 5080.
735. G. M. Chaban, J. O. Jung and R. B. Gerber, *J. Phys. Chem*., **104**, 2000, 10035.
736. E. Kassab, J. Langlet, E. Evgleth and Y. Akacem, *THEOCHEM*, **53**, 2000, 267.
737. L. Rodriguez-Santiago, M. Sodupe, A. Oliva and J. Bertran, *J. Phys. Chem*., **104**, 2000, 1256.
738. O. Bludsky, J. Chocholousova, J. Vacek, F. Huisken and P. Hobza, *J. Chem. Phys*., **113**, 2000, 4629.
739. R. A. J. O'Hair, P. S. Broughton, M. L. Styles, B. T. Frink and C. M. Hadad, *J. Am. Soc. Mass Spectrom*., **11**, 2000, 687.
740. G. Raabe, *Z. Naturforsch*., *A*: *Phys. Sci*., **55**, 2000, 609.
741. L. Gontrani, B. Mennucci and J. Tomasi, *THEOCHEM*, **500**, 2000, 113.
742. F. Rogalewic and Y. Hoppilliard, *Int. J. Mass Spectrom*., **199**, 2000, 235.
743. P. Bandyopadhyay and M. S. Gordon, *J. Chem. Phys*., **113**, 2000, 1104.
744. S. G. Stepanian, *Biopolim. Kletka*, **15**, 1999, 374.
745. M. A. Berg, S. J. Salpietro, A. Perczel, O. Farkas and I. G. Csizmadia, *THEOCHEM*, **504**, 2000, 127.
746. W. D. Arnold, L. K. Sanders, M. T. McMahon, A. V. Volkov, G. Wu, P. Coppens, S. R. Wilson, N. Godbout and E. Oldfield, *J. Am. Chem. Soc*., **122**, 2000, 4708.
747. R. Berger and M. Quack, *ChemPhysChem*, **1**, 2000, 57.
748. J. K. Laerdahl, R. Wesendrup and P. Schwerdtfeger, *ChemPhysChem*, **1**, 2000, 60.

749. D. C. Chatfield and S. E. Wong, *J. Phys. Chem., B*, **104**, 2000, 11342.
750. P. A. Nielsen, P.-O. Norrby, T. Liljefors, N. Rega and V. Barone, *J. Am. Chem. Soc.*, **122**, 2000, 3151.
751. J. M. Mercero, P. Barrett, C. W. Lam, J. E. Fowler, J. M. Ugalde and L. G. Pedersen, *J. Comput. Chem.*, **21**, 1999, 43.
752. M. Noguera, L. Rodriguez-Santiago, M. Sodupe and J. Bertran, *THEOCHEM*, **537**, 2001, 307.
753. F. Rogalewicz, G. Ohanessian and N. Gresh, *J. Comput. Chem.*, **21**, 2000, 963.
754. R. M. Minyaev, A. G. Starikov and V. I. Minkin, *Mendeleev Commun.*, **10**, 2000, 43.
755. R. C. Dundar, *J. Phys. Chem.*, **104**, 2000, 8067.
756. T. Marino, N. Russo and M. Toscano, *J. Inorg. Biochem.*, **79**, 2000, 179.
757. R. J. Lavrich and M. J. Tubergen, *J. Am. Chem. Soc.*, **122**, 2000, 2938.
758. A. D. Headley and S. D. Starnes, *Trends Org. Chem.*, **7**, 1998, 75.
759. A. D. Headley and S. D. Starnes, *J. Comput. Chem.*, **21**, 2000, 426.
760. A. D. Headley and S. D. Sarnes, *THEOCHEM*, **507**, 2000, 281.
761. A.K. Fuzery and I. G. Csizmadia, *THEOCHEM*, **501–2**, 2000, 539.
762. I. Jakli, A. Perczel, O. Farkas, A. G. Csaszar, C. Sosa and I. G. Csizmadia, *J. Comput. Chem.*, **21**, 2000, 626.
763. S. Ro and C.-J. Yoon, *Z. Phys. Chem.* (*Muenchen*), **214**, 2000, 1699.
764. I. S. Scarminio, W. J. Barreto, D. N. Ishikawa, E. L. Paczkowski and I. C. Arruda, *Anal. Sci.*, **16**, 2000, 1337.
765. C. Cheng and S.-C. Wu, *J. Chromatogr., A*, **896**, 2000, 299.
766. T. Oikawa, M. Watanabe, H. Makiura, H. Kusakabe, K. Yamade and K. Soda, *Biosci. Biotechnol, Biochem.*, **63**, 1999, 2168.
767. L. Wei and W. D. Lubell, *Org. Lett.*, **2**, 2000, 2595.
768. M. J. Hateley, D. A. Schichl, H.-J. Kreuzfeld and M. Beller, *Tetrahedron Lett.*, **41**, 2000, 3821.
769. M. Hasegawa, D. Taniyama and K. Tomioka, *Synlett*, **1**, 2001, 25.
770. E. Andersson and G. Nils, *Origins Life Evol. Biosphere*, **30**, 2000, 9.
771. J. Douda and V. A. Basiuk, *J. Anal. Appl. Pyrolysis*, **56**, 2000, 113.
772. H.-X. Wang, X.-C.Yan, M.-D. Luo and S.-S. Qu, *Hunan Daxue Xuebao Ziran Kexueban*, **27**, 2000, 32.
773. L. Z. Cai, Y. R. Chen, G. J. Zhang, Y. Q. Jia, H. S. Qian, S. K. Ruan and J. F. Duan, *Thermochim. Acta*, **345**, 2000, 53.
774. H. N. Gopi and V. V. S. Babu, *J. Pept. Res.*, **55**, 2000, 295.
775. G. Sennyey, H. X. Hai, L. Olivier and E. Cheylan, *Pept.* 1988, *Proc. Eur. Pept. Symp., 25th*, 1999, 244.
776. Z. Schmel and Z. Kupihar, *Molecules*, **5**, 2000, M162.
777. J. E. Sheppeck, H. Kar and H. Hong, *Tetrahedron Lett.*, **41**, 2000, 5329.
778. M. Balse, H. J. Kim, G. Han and V. J. Hruby, *J. Pept. Res.*, **56**, 2000, 70.
779. C. Carreno, M. E. Mendez, Y.-D. Kim, H.-J. Kim, S. A. Kates, D. Andreu and F. Albericio, *J. Pept. Res.*, **56**, 2000, 63.
780. B. H. Hu and P. B. Messersmith, *Tetrahedron Lett.*, **41**, 2000, 5795.
781. K. Ananda, G.-R. Vasanthakumar and V. V. S. Babu, *Protein Pept. Lett.*, **8**, 2001, 45.
782. L. S. Sin, T. Lanza, S. E. de Laszlo, Q. Truong, T. Kamenecka and W. K. Hagmann, *Tetrahedron Lett.*, **41**, 2000, 7013.
783. J. Xu and J. Yang, *Huaxue Tongbao*, 2000, 26; *Chem. Abs.*, **132**, 293986.
784. H.-W. Shao, Y. Wu and R. Li, *Synth. Commun.*, **30**, 2000, 1911.
785. D. Albanese, D. Landini, V. Lupi and M. Penso, *Eur. J. Org. Chem.*, 2000, 1443.

786. C. Tao and Q. Lun, *Huaxue Shiji*, **22**, 2000, 120; *Chem. Abs*., **133**, 164293.
787. Q. He, *Diss. Abstr. Int. B*, **61**, 2000, 595.
788. M. S. Nair, S. T. David and M. Anbu, *Indian J. Chem. Sect. A*, **38A**, 1999, 850.
789. M. S. Nair, S. T. D. Manickam, *J. Indian Chem. Soc*., **76**, 1999, 472.
790. M. S. Islam, M. N. Islam and H. N. Roy, *J. Bangladesh Chem. Soc*., **11**, 1998, 9.
791. H. Singh and A. K. Varshney, *ACGC Chem. Res. Commun*., **9**, 1999, 9.
792. K.-M. Yao, W. Zhou, G. Lu and L.-F. Shen, *Huaxue Xuebao*, **58**, 2000, 1275.
793. W. Chobpattana, I. J. Jeon and J. S. Smith, *J. Agric. Food Chem*., **48**, 2000, 3885.
794. N. P. Kuznetsova, R. N. Mishaeva, O. A. Pisarev and L. R. Gudkin, *Zh. Prikl. Khim.* (*S.-Petersburg*), **73**, 2000, 796.
795. V. I. Beketov, R. D. Voronina, D. G. Filatova and N. B. Zorov, *J. Anal. Chem*., **55**, 2000, 1148.
796. H. L. Singh, M. Sharma and A. K. Varshney, *Synth. React. Inorg. Met.-Org. Chem*., **30**, 2000, 445.
797. M. R. Kembhavi, A. l. Harihar and S. T. Nandibewoor, *Oxid. Commun*., **23**, 2000, 304.
798. A. L. Harihar, M. R. Kembhavi and S. T. Nandibewoor, *Indian J. Chem. Sect.A*: *Inorg*., *Bio-inorg*., *Phys*., *Theor. Anal. Chem*., **39A**, 2000, 769.
799. M. S. R. Kembhavi, R. G. Panari and S. T. Nandibewoor, *Inorg. React. Mech*., **1**, 1999, 225.
800. M. Wagner and H. Kunz, *Synlett*, 2000, 400.
801. P. Strazzolini, M. Scuccato and A. G. Giumanini, *Tetrahedron*, **56**, 2000, 3625.
802. L. A. Barnhurst, Y. Wan and A. G. Kutateladze, *Org. Lett*., **2**, 2000, 799.
803. V. V. Babu, H. N. Gopi and K. Ananda, *Indian J. Chem. Sect. B*: *Org. Chem*., *Incl. Med. Chem*., **39B**, 2000, 291.
804. B. T. Gowda, K. Geetha and K. Jyothi, *Oxid*., *Commun*., **23**, 2000, 274.
805. F. H. Khan and F. Ahmad, *Oxid. Commun*., **23**, 2000, 285.
806. B. T. Gowda, S. D. Quine, P. S. K. Krishna, *J. Indian Chem. Soc*., **77**, 2000, 413.
807. A. Bote, R. Hernandez and E. Suarez, *J. Org. Chem*., **65**, 2000, 4930.
808. I. Akichika, T. Kodama, S. Inagaki and Y. Masaki, *Chem. Lett*., **5**, 2000, 542.
809. E.-M. Ungureanu, G. Stanciu and S. I. Rosca, *Sci. Bull.* '*Politeh*' *Univ. Bucharest*, *Ser. B*., **61**, 2000, 5; *Chem. Abs*., **133**, 4912.
810. M. J. Snider and R. Wolfenden, *J. Am. Chem. Soc*., **122**, 2000, 11507.
811. D. A. Quagliato, P. M. Andrae and E. M. Matelan, *J. Org. Chem*., **65**, 2000, 5037.
812. M. Groarke, M. A. McKervey and M. Nieuwenhuyzen, *Tetrahedron Lett*., **41**, 2000, 1275.
813. M. Groarke, M. A. McKervey, H. Moncrieff and M. Nieuwenhuyzen, *Tetrahedron Lett*., **41**, 2000, 1279.
814. P. Darkins, M. Groake, M. A. McKervey, H. M. Moncrieff, N. McCarthy and M. Nieuwenhuyzen, *J. Chem. Soc*., *Perkin Trans. 1*, 2000, 381.
815. K. Fan, P. Ouyang and X. Wu, *Nanjing Huagong Daxue Xuebao*, **22**, 2000, 42; *Chem. Abs*. **133**, 252695.
816. N. Ahmad, M. A. J. Miah, M. R. Haque and M. M. Ali, *J. Polym. Mater*., **17**, 2000, 283.
817. A. L. Harihar, M. Kembhavi and S. T. Nandibewoor, *Monatsh. Chem*., **131**, 2000, 739.
818. V. R. Chourey, S. Pandey, L. V. Shastry and V. R. Shastry, *Chim. Acta Turc*., **26**, 1998, 21.
819. S. Pandey, V. J. Chourey, L. V. Shastry and V. R. Shastry, *Oxid. Commun*., **23**, 2000, 138.

820. B. T. Gowda, P. J. M. Rao and S. P. Nayak, *Oxid. Commun.*, **23**, 2000, 459.
821. O. Merwitz, H.-M. Nitzsche and E. Hochhauser, *Isot. Environ. Health Stud.*, **34**, 1998, 319.
822. B.-C. Chen, A. P. Skoumbourdis, P. Guo, M. S. Bednarz, O. R. Kocy, J. E. Sundeen and G. D. Vite, *J. Org. Chem.*, **64**, 1999, 9294.
823. M. Kawase, M. Hirabayashi, H. Kumakura, S. Saito and K. Yamamoto, *Chem. Pharm. Bull.*, **48**, 2000, 114.
824. T. Kawabata, H. Suzuki, Y. Nagae and K. Fuji, *Angew. Chem., Int. Ed.*, **39**, 2000, 2155.
825. K. Miyashita, H. Miyabe, K. Tai, C. Kurozumi, H. Iwaki and T. Imanishi, *Tetrahedron*, **55**, 1999, 12109.
826. T. Kawabata, H. Suzuki, Y. Nagae, T. Wirth, K. Yahiro and K. Fuji, *Proc. ECSOC-3; Proc. ECSOC*-4, 1999–2000, 1126; *Chem. Abs.*, 2001, 138637.
827. M. Kawase, S. Saito and T. Kurihawa, *Chem. Pharm. Bull.*, **48**, 2000, 1338.
828. K. Kichuchi, R. Ansai and K. Tanaka, *Seibunkaisei Kemikarasu to Purasuchikku*, 2000, 9.
829. M. Saito, T. Yamamoto and H. Fukuda, *Seibunkaisei Kemikarasu to Purasuchikku*, 2000, 27.
830. K. Matsuoka, *Seibunkaisei Kemikarasu to Purasuchikku*, 2000, 36.
831. L. M. Chabaka, A. Y. Allam and G. A. M. Nawwar, *Z. Naturforsch. B. Chem. Sci.*, **55**, 2000, 104.
832. A. J. Phillips, Y. Uto, P. Wipf, M. J. Reno and D. R. Williams, *Org. Lett.*, **2**, 2000, 1165.
833. G. Dewynter, A. H. Hajri, L. Toupet and J.-L. Montero, *Synth. Commun.*, **30**, 2000, 2541.
834. A. Wahhab and J. Leban, *Tetrahedron Lett.*, **41**, 2000, 1487.
835. G. S. Gadaginamath, R. R. Kavali, S. A. Patil and A. S. Shyadligeri, *Indian J. Chem. Sect B*, **38B**, 1999, 1123.
836. E. Okada and N. Tsukushi, *Heterocycles*, **53**, 2000, 127.
837. J. Hao, H. Ohkura, H. Amii and K. Uneyama, *Chem. Commun.*, 2000, 1883.
838. F. Dujols and M. Mulliez, *Phosphorus, Sulfur Silicon Relat. Elem.*, **157**, 2000, 165.
839. T. Brinkman, A. Gilgf, A. Hamm, H. Lusch, G. Morbach and H. C. Uzar, *Tetrahedron: Asymmetry*, **11**, 2000, 3827.
840. J. Cheng, J. W. Ziller and T. J. Deming, *Org. Lett.*, **2**, 2000, 1943.
841. I. Shin, M.-R. Lee, J. Lee, M. Jung, W. Lee and J. Yoon, *J. Org. Chem.*, **65**, 2000, 7667.
842. A. Boto, R. Hernandez and E. Suarez, *Tetrahedron Lett.*, **41**, 2000, 2899.
843. S. Ledoux, J.-P. Celerier and G. L. Hommet, *Tetrahedron Lett.*, **40**, 1999, 9019.
844. M. M. Garazd, Y. L. Garazd, M. N. Smirnov and V. P. Khilya, *Chem. Nat. Compd.*, **35**, 2000, 415.
845. M. Hasan, F. Shaheen, U. Yunus and S. Munir, *Turk. J. Chem.*, **24**, 2000, 165.
846. G. R. Sridhar, V. S. Murty, S. H. Lee, I. A. Blair and T. M. Penning, *Tetrahedron*, **57**, 2001, 407.
847. A.-X. Yan, G.-W. Xing, Y.-H. Ye, G.-L. Tian, M.-S. Wong and K.-S. Lee, *Tetrahedron Lett.*, **41**, 2000, 5379.
848. N. Raos and L. Zuza, *Kem. Ind.*, **49**, 2000, 45.
849. Y. Ito, M. Gerz and Y. Nakahara, *Tetrahedron Lett.*, **41**, 2000, 1039.
850. M. A. Abbady and S. H. Abdel-Hafez, *Phophorus, Silicon Sulfur Relat. Elem.*, **160**, 2000, 121.
851. C. Aliaga and E. A. Lissi, *Can. J. Chem.*, **78**, 2000, 1052.

852. A. Khazaei, M. R. Zamani and E. Mehdipour, *Asian J. Chem.*, **12**, 2000, 327.
853. S. Kitagaki, M. Kinoshita, M. Takeba, M. Anada and S. Hashimoto, *Tetrahedron; Asymmetry*, **11**, 2000, 3855.
854. J. Baraniak, R. Kaczmarek and W. J. Stec, *Tetrahedron Lett.*, **41**, 2000, 9139.
855. S. Boudjabi, G. Dewynter and J.-L. Montero, *Synlett*, 2000, 716.
856. E. Alonso, E. Alonso, A. Solis and C. Del Pozo, *Synlett*, 2000, 698.
857. K. T. Sprott and P. R. Hanson, *J. Org. Chem.*, **65**, 2000, 4721.
858. I. Devedjiev, K. Petrova and I. Glavchev, *Synth. Commun.*, **30**, 2000, 4411.
859. M. C. Pirrung, K. D. James and V. P. Rana, *J. Org. Chem.*, **65**, 2000, 8448.
860. E. Modica, R. Zanaletti, M. Freccero and M. Mella, *J. Org. Chem.*, **66**, 2001, 41
861. C. Cai, A. Vadim and V. J. Hruby, *J. Org. Chem.*, **66**, 2001, 1339.
862. D. Gryko and J. Janusz, *Helv. Chim. Acta*, **83**, 2000, 2705.
863. K. Cejpek, J. Valusek and J. Velisek, *J. Agric. Food Chem.*, **48**, 2000, 3560.
864. L. F. Krylova, *Khim. Interesakh Ustoich Razvit.*, **7**, 1999, 465.
865. D. J. Black, G. W. V. Cave, D. P. Lydon and J. P. Rourke, *J. Organomet. Chem.*, **601**, 2000, 305.
866. E. M. Shoukry, M. Shoukry, A. E. Mahgoub, H. M. Galal, *Ann. Chim.* (*Rome*), **90**, 2000, 593.
867. M. Q. Ehsan, M. Jebin, E. Ahmed and A. M. Quyser, *Indian J. Chem. Sect. A*: *Inorg. Bio-inorg. Phys. Theor. Anal. Chem.*, **38A**, 1999, 826.
868. O. Brosch, T. Weyhermeuller and N. Metzler-Nolte, *Inorg. Chem.*, **38**, 1999, 5308.
869. U. Kazmaier, D. Schauss, S. Raddatz and M. Pohlman, *Chem. Eur. J.*, **7**, 2001, 456.
870. M. Adamczyk, R. E. Reddy and S. D. Rege, *Synth. Chem. Commun.*, **30**, 2000, 1389.
871. Y. Du, H. Deng and S. Zhong, *Sichuan Daxue Xuebao Ziran Kexueban*, **37**, 2000, 79.
872. A. S. Kazachenko, E. V. Legler, O. V. Per'yanova and Y. A. Vstavskaya, *Pharm. Chem. J.*, **33**, 1999, (publ. 2000) 470.
873. J. Liu, J. Peng, E. Wang, L. Bi and S. Guo, *J. Mol. Struct.*, **525**, 2000, 71.
874. F. Ghazrib, A. S. Sadjadi and F. Zourofi, *Rev. Inorg. Chem.*, **20**, 2000, 219.
875. D. Wen, Y. Liu and S. Qu, *Huaqiao Daxue Xuebao*, *Ziran Kexueban*, **21**, 2000, 84; *Chem. Abs.*, 2000, 245781.
876. A. K. Das, *Inorg. React.*, *Mech.* (*Amsterdam*), **1**, 2000, 309.
877. L. B. Saran and M. N. Srivastava, *Rev. Roum. Chim.*, **44**, 1999, 125.
878. C. S. Dexter, R. F. W. Jackson and J. Elliott, *Tetrahedron*, **56**, 2000, 4539.
879. N. A. Dobrynina, M. E. Akat'eva and L. S. Nikolaeva, *Zh. Fiz. Khim.*, **74**, 2000, 942.
880. S. Hanessian and M. Bayrakdarian, *Bioorg. Med. Chem. Lett.*, **10**, 2000, 427.
881. Y. Kawanam, I. T. Mitsuie, M. Miki, T. Sakamoto and K. Nishitani, *Tetrahedron*, **56**, 1999, 175.
882. R. Hassa and J. O. Dziegielewski, *Spectrosc. Biol. Mol.: New Dir. Eur. Conf.* 8*th*, 1999, 23; *Chem. Abs.*, **133**, 110533y.
883. A. A. Goodwin, D. Xianhui and T. J. Deming, *J. Organomet. Chem.*, **589**, 1999, 111.
884. X.-M. Gao, Y.-H. Ye, G.-L. Tian and D. Wang, *Pept. Biol. Chem. Proc. Chin. Pept. Symp.* 5*th*, 1998, (publ. 2000), 33 *Chem. Abs.*, **134**, 131782q.
885. J. F. Read, S. A. Bewick, C. R. Graves, J. McPherson, J. C. Salah, A. Theriault and A. E. H. Wyand, *Inorg. Chim. Acta*, **303**, 2000, 244.
886. Puttaswamy and N. M. M. Gowda, *Trans. Ill. State Acad. Sci.*, **93**, 2000, 115.
887. M. S. R. Kembhavi, R. G. Panari and S. T. Nandibewoor, *J. Saudi Chem. Soc.*, **3**, 1999, 121.
888. A. K. Kataria and B. L. Hiran, *Asian J. Chem.*, **12**, 2000, 816.
889. H. Iloukhani, N. Rashidi and M. Moghadasi, *Asian J. Chem.*, **12**, 2000, 1209.
890. S. Dubey and A. Pandey, *Oxid. Commun.*, **23**, 2000, 125.

891. S. Dubey and A. Pandey, *Oxid. Commun.*, **23**, 2000, 443.
892. S. Dubey and A. Pandey, *Oxid. Commun.*, **23**, 2000, 451.
893. M. Choubey, S. Dubey and A. Pandey, *J. Indian Counc. Chem.*, **15**, 1998, 71.
894. M. Choubey, S. Dubey and A. Pandey, *J. Inst. Chem.* (*India*), **71**, 1999, 49.
895. V. S. R. Raju, P. K. Sharma and K. K. Banerji, *Indian J. Chem. Sect. A*: *Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, **39A**, 2000, 650.
896. H. Gangwani, P. K. Sharma and K. K. Banerji, *Indian J. Chem. Sect. A*: *Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, **39A**, 2000, 436.
897. D. Perrin and W. H. Koppenol, *Arch. Biochem. Biophys.*, **377**, 2000, 266.
898. S. Y. Men'shikov, A. V. Vurasko, L. S. Molochnikov, E. G. Kovalyova and A. A. Efendiev, *J. Mol. Catal. A*: *Chem.*, **158**, 2000, 447.
899. A. Hanaki, T. Kinoshita and T. Ozawa, *Bull. Chem. Soc. Jpn.*, **73**, 2000, 73.
900. F. Huerta, E. Morallon, C. Quijada, J. L. Vazquez and L. E. A. Berlouis, *J. Electroanal. Chem.*, **489**, 2000, 92.
901. A. Brazill, P. Singhal and W. G. Kuhr, *Anal. Chem.*, **72**, 2000, 5542.
902. G. Ionita, V. E. Sahini, G. Semenescu and P. Ionita, *Acta Chim. Slov.*, **47**, 2000, 111.
903. T. Kotiaho, M. N. Eberlin, P. Vainiotalo and R. Kostiainen, *J. Am. Mass Spectrom.*, **11**, 2000, 526.
904. R. S. Oakes, A. A. Clifford, K. D. Bartle, M. Thornton-Pett and C. M. Rayner, *Chem. Commun.* (*Cambridge*), 1999, 247.
905. M. Falorni, G. Giacomelli, A. Porcheddu and M. Taddei, *J. Org. Chem.*, **64**, 1999, 8962.
906. B. Sun, G. Tian, W. Wang, N. Jin and Y. Ye, *Beeijing Daxue Xuebao Ziran Kexueban*, **35**, 1999, 441.
907. F. Yuste, B. Ortiz, A. Carrasco, M. Peralta, L. Quintero, R. Sanchez-Obregon, F. Walls and J. L. Garcia Ruano, *Tetrahedron*: *Asymmetry*, **11**, 2000, 3079.
908. D. Berkes, A. Kolarovic, F. Povazanec, *Tetrahedron Lett.*, **41**, 2000, 5257.
909. A. Assmann, M. Bonifacic, K. Briviba, H. Sies and K.-D. Asmus, *Free Radical Res.*, **32**, 2000, 371.
910. X. Li, R. Lou, C.-H. Yeung, A. C. S. Chan and W. K. Wong, *Tetrahedron*: *Asymmetry*, **11**, 2000, 2077.
911. F. Robert and D. Sinou, *J. Organomet. Chem.*, **604**, 2000, 99.
912. R. Giernoth, H. Heinrich, N. J. Adams, R. J. Deeth, J. Bargon and J. M. Brown, *J. Am. Chem. Soc.*, **122**, 2000, 12381.
913. D. A. Perrey and F. M. Uckun, *Tetrahedron Lett.*, **42**, 2001, 1859.
914. E. A. Yelin, V. V. Onoprienko, I. A. Kudelina and A. I. Miroshnikov, *Russ. J. Bioorg.*, **26**, 2000, 774.
915. C. Douat, A. Heitz and J. Martinez, *Tetrahedron Lett.*, **41**, 2000, 37.
916. R. Muller, H. Rottele, H. Henke and H. Waldmann, *Chem. Eur. J.*, **6**, 2000, 2032.
917. M. Bonifacic, D. A. Armstrong, I. Carmichael, and D.-K. Asmus, *J. Phys. Chem. B*, **104**, 2000, 643.
918. M. Lodder, C. F. Crasto, A. L. Laikhter, H. An, T. Arslan, V. A. Karginov, G. F. Short lll and S. M. Hecht, *Can. J. Chem.*, **78**, 2000, 884.
919. M.-L. Wang, C.-C. Ou and J.-J. Jwo, *Chem. Eng. Commun.*, **197**, 2000, 233.
920. G. C. A. M. Vanhaelewyn, S. A. Amira, W. K. P. G. Mondelaers and F. J. Callens, *Spectrochim. Acta Part A*, **56A**, 2000, 387.
921. B. Rakvin and N. Maltar-Strmecki, *Spectrochim. Acta Part A*, **56A**, 2000, 399.
922. A. A. Bugay, V. A. Onischuk, T. L. Petrenko and V. V. Telslenko, *Appl. Radiat. Isot.*, **52**, 2000, 1189.
923. Y.-J. Cherng, *Tetrahedron*, **56**, 2000, 8287.

924. Y. Xu, Q. Song, X. Ma, W. Wang and S. Yao *Fushe Yanjiu Yu Fushe Gongi Xuedao*, **17**, 1999, 239.
925. S. Ikeda, S. Murata, K. Ishii, H.-O. Hamaguchi, *Bull. Chem. Soc. Jpn*., **73**, 2000, 2783.
926. Q. Song, Q. Li, H. Sun, Y. Xu, C. Chen, S. Yu, X. Ma, S. Yao and N. Lin, *Prog. Nat. Sci*., **10**, 2000, 314.
927. Q. Song, Y. Xu, S. Yu, C. Chen, X. Ma, W. Wang, S. Yao and N. Lin, *Sci. China Ser. B*: *Chem*., **42**, 1999, 561.
928. G. L. Hug, K.-D. Asmus and D. Armstrong, *J. Phys. Chem. B*, **104**, 2000, 6674.
929. J. Zaccaro, J. Matic, A. S. Myerson and B. A. Garetz, *Cryst. Growth Des*., **1**, 2000, 5.
930. H. S. Knowles, K. Hunt and A. F. Parsons, *Tetrahedron Lett*., **41**, 2000, 7121.
931. C. Grewer, J. Jaeger, B. K. Carpenter and G. P. Hess, *Biochemistry*, **39**, 2000, 2063.
932. C.T. Houston and J. P. Reilly, *J. Phys. Chem*., **104**, 2000, 10383.
933. S. A. Serron, W. S. Aldridge, R. M. Danell and T. J. Meyer, *Tetrahedron Lett*., **41**, 2000, 4039.
934. C. Easton, M. C. Merrett and P. Razzino, *J. Chem. Soc. Perkin Trans. 2*, 2000, 693.
935. H. Nishino, A. Kosaka, G. A. Hembury, H. Shitomi, H. Onuki and Y. Inoue, *Org. Lett*., **3**, 2001, 921.
936. S. Pagliari, R. Corradini, G. Galaverna, S. Sforza, A. Dossena and R. Marchelli, *Tetrahedron Lett*., **41**, 2000, 3681.
937. S. Santra, P. Zhang, and W. J. Tan, *J. Phys. Chem*., **104**, 2000, 12021.
938. K. Ruan, S. Tian, R. Lange and C. Balny, *Biochem. Biophys. Res. Commun*., **269**, 2000, 681.
939. S. V. Galushko, *Aminophosphonic Aminophosphinic Acids*, 2000, 205.
940. Z. H. Kudzin and M. Sochacki, *Aminophosphonic Aminophosphinic Acids*, 2000, 363.
941. M. L. Bui, D. H. Le, Q. N. Huynh, Q. B. Ngo and T. T. V. Tran, *Tap Chi Hao Hoc*, **38**, 2000, 35.
942. P. Capitan, T. Malmezat, D. Breuille and C. Obled, *J. Chromatogr*., *B. Biomed. Sci Appl*., **732**, 1999, 127.
943. W. Kulik, R. M. Kok, K. de Meer and C. Jakobs, *J. Chromatogr*., *B*: *Biomed. Csi. Appl*., **738**, 2000, 99.
944. A. V. Kaznachneev, O. N. Khokhlova, V. F. Selemenov, V. Y. Khokhlov and N. Y. Mokshina, *J. Anal. Chem*., **55**, 2000, 335.
945. I. Molnar-Perl and Z. F. Katona, *Chromatographia*, **51(Suppl)**, 2000, S228.
946. G. S. Nadiger and U. D. Bongale, *Man-made Text. India*, **42**, 1999, 305.
947. I. G. Casella, M. Gatta and T. R. I. Cataldi, *J. Chromatogr*., *A*, **878**, 2000, 57.
948. F. N. Kaputskii, T. L. Yurkshtovich, G. L. Starobinets, P. M. Bychkovskii and T. I. Borshchenskaya, *Zh. Fiz. Khim*., **74**, 2000, 277.
949. A. V. Kaznacheev, O. N. Khokhlova, V. F. Selemenev and V. Y. Khokhlov, *Zh. Fiz. Khim*., **74**, 2000, 283.
950. I. V. Aristov, V. Y. Khokhlov, O. N. Khokhlova, O. V. Bobreshova and V. F. Selemenev, *Zh. Fiz. Khim*., **74**, 2000, 288.
951. V. I. Vasil'eva and T. V. Eliseeva, *Russ. J. Electrochem*., **36**, 2000, 30.
952. A. Zammouri, S. Chanel, L. Muir and G. Grevillot, *Ind. Eng. Chem. Res*., **39**, 2000, 1397.
953. A. Zammouri, L. Muhr and G. Grevillot, *Ind. Eng. Chem. Res*., **39**, 2000, 2468.
954. J. Liu, G. Liu H. Yan, X. Cheng, B. He, G. Jiang and G. Yang, *Lizi Jiaohuan Yu Xifu*, **16**, 2000, 521; *Chem. Abs*., 2001, 39433.
955. A. Mohammad, S. Anwar and V. Agrawal, *J. Indian Chem. Soc*., **76**, 1999, 452.

956. Q.-H. Zhu, B. Xiong, Q.-Y. Deng and L.-M. Zeng, *Fenxi Ceshi Xuebao*, **19**, 2000, 8; *Chem. Abs.* , **133**, 232163.
957. Z. H. Kudzin, M. Saganiak, A. Kotynski and W. Ciesielski, *J. Chromatogr.*, *A*, **888**, 2000, 335.
958. G. Ionita, *J. Planar Chromatog.-Mod. TLC*, **12**, 1999, 421.
959. *Food Sci. Technol.* (*NY*), 100 (*Food Analysis by HPLC*), 2000, 55.
960. K. Harada and K. Fujii, *Farumashia*, **13**, 2000, 443.
961. M. Yang and S. A. Tomellini, *Anal. Chim. Acta*, **409**, 2000, 45.
962. K. Sato, J.-Y. Jin, T. Takeuchi, T. Miwa, Y. Takekoshi, S. Kanno and K. Susumu, *Analyst* (*Cambridge*, *UK*), 125, 2000, 1041.
963. I. N. Krasnova, L. A. Kartsova and Y. V. Cherkas, *J. Anal. Chem.*, **55**, 2000, 58.
964. X. Ling, Y.-Q. Liu, M. Du and B. Xu, *Shenyang Yaoke Daxue Xuebao*, **17**, 2000, 349; *Chem. Abs.*, 2000, 750616.
965. T. Miyazawa, H. Minowa, Y. Shindo and T. Yamada, *J. Liq. Chromatogr. Relat. Technol.*, 23, 2000, 1061.
966. M. Schlauch and A. W. Frahm, *J. Chromatogr.*, **868**, 2000, 197.
967. E. Tesarova, Z. Bosakova and I. Zuskova, *J. Chromatogr.*, *A*, **875**, 2000, 147.
968. A. Berthod, X. Chen, J. P. Kullman, D. W. Armstrong, F. Gasparrini and I. D'Acquarica, *Anal. Chem.*, **72**, 2000, 1767.
969. K. Chen, Y.-Q. Chen, X.-R. Xu, Y.-J. Yu, Z.-Q. Guo and X.-M. Jiang, *Sepu*, **18**, 2000, 14, *Chem. Abs.*, **132** 273568
970. W. Lee, *Anal. Lett.*, **33**, 2000, 347.
971. M. Schlauch, F.-J. Volk, K. P. Fondekar, J. Wede and A. W. Frahm, *J. Chromatogr.*, *A*, **897**, 2000, 145.
972. A. Peter, G. Torok, G. Toth and W. Lindner, *J. High Resolut. Chromatogr.*, **23**, 2000, 628.
973. M. M. Or-Rashid, R. Onodera, S. Wadud and N. Mohammed, *J. Chromatogr.*, *B*: *Biomed. Sci. Appl.*, **741**, 2000, 279.
974. Y.-Y. Zhao, H. Wang and H.-S. Zhang, *Fenxi Kexue Xuebao*, **16**, 2000, 188; *Chem. Abs.*, 2000, 565680.
975. B.-H. Fu, X.-H. Zhou and D.-M. Xiong, *Chongqing Daxue Xuebao*, *Ziran Kexueban*, **23**, 2000, 83; *Chem. Abs.*, **134**, 125343.
976. N. Kiba, G. Shimura. H. Hayashi, M. Tachibana, K. Tani, H. Koizumi and Y. Hibibo, *Anal. Sci.*, **16**, 2000, 981.
977. H. Kanazawa, T. Sunamoto, Y. Matsushima. A. Kikuchi and T. Okano, *Anal. Chem.*, **72**, 2000, 5961.
978. B. Polak and W. Golkiewicz, *J. Liq. Chromatogr. Rel. Technol.*, **23**, 2000, 2807.
979. K. Chen, Y.-Q. Chen, X.-R. Xu, Y.-J. Yu, Z.-Q. Guo and X.-M. Jiang, *Sepu*, **18**, 2000, 14; *Chem. Abs.*, **132**, 273568.
980. J. Lichfield, E. Verpoote and N. F. De Rooij, *Electrophoresis*, **22**, 2001, 258.
981. Q.-H. Ru, J. Yao, G.-A. Luo, Y.-X. Zhang and C. Yan, *J. Chromatogr.*, *A*, **894**, 2000, 337.
982. J. Wang, M. P. Chatrathi and B. Tian, *Anal. Chem.*, **72**, 2000, 5774.
983. L. Ceriotti, E. Verpoorte and N. F. De Rooij, *Micro Total Anal. Syst.* 2000, *Proc. μTAS Symp.*, 4*th*, 2000, 225; *Chem. Abs.*, **133**, 260904.
984. S.-l. Zhai, J.-G. Liu and G.-S. Luo, *Gaoxiao Huaxue Gongcheng Xuebao*, **14**, 2000, 293; *Chem. Abs.*, **133**, 275695.
985. S. L. Zhai, G. S. Luo and J. G. Liu, *Sep. Purif. Technol.*, **21**, 2001, 197.
986. G. L. DeVault and M. J. Sepianik, *J. Microcolumn Sep.*, **12**, 2000, 419.
987. N. J. Munro, Z. Huang, D. N. Finegold and J. P. Landers, *Anal. Chem.*, **72**, 2000,

2765.
988. W. Zhan, T. Wang and S. F. Y. Li, *Electrophoresis*, **21**, 2000, 3593.
989. F. Dang and Y. Chen, *Sci. China Ser. B: Chem.*, **42**, 1999, 663.
990. K. Tsukagoshi, M. Otsuka, M. Hashimoto, R. Nakajima and H. Kimoto, *Chem. Lett.*, 2000, 98.
991. Y. Zhang and F. A. Gomez, *Electrophoresis*, **21**, 2000, 3305.
992. E. Zahou, H. Jornvall and T. Bergman, *Anal. Biochem.*, **281**, 2000, 115.
993. C. Perrin, M. Vargus, Y. Van der Heyden, M. Maftouh and D. L. Massart, *J. Chromatogr.*, **883**, 2000, 249.
994. E. Tobler, M. Lammerhofer and W. Lindner, *J. Chromatogr., A*, **875**, 2000, 341.
995. V. Piette, M. Fillet, W. Lindner and J. Crommen, *J. Chromatogr., A*, **875**, 2000, 353.
996. M. G. Schmid, N. Grobuschek, C. Tuscher, G. Gubitz, A. Vegvari, E. Machtejevas, A. Maruska and S. Hjerten, *Electrophoresis*, **21**, 2000, 3141.
997. T. Horimai, T. Arai and Y. Sato, *J. Chromatogr., A*, **875**, 2000, 295.
998. E. Tesarova, Z. Bosakova and I. Zuskova, *J. Chromatogr., A*, **875**, 2000, 147.
999. V. Piette, W. Lindner and J. Crommen, *J. Chromatogr., A.*, **894**, 2000, 63.
1000. R. M. Latorre, J. Saurina and S. Hernandez-Cassou, *Electrophoresis*, **21**, 2000, 563.
1001. R. M. Latorre. J. Saurina and S. Hernandez-Cassou, *J. Chromatogr., A*, **871**, 2000, 331.
1002. M. I. Amores-Sanchez and M. A. Medina, *Clin. Chem. Lab. Med.*, **38**, 2000, 199.
1003. A. H. B. Wu, V. Holtman, F. S. Apple, V. Ricciuti, P. M. Di-Bello, D. Jacobsen, *Ann. Clin. Lab. Sci.*, **30**, 2000, 185.
1004. J.-l. Zhan, Z.-J. Zhu and D.-l. Jiang, *Fenxi Ceshi Xuebao*, **19**, 2000, 72; *Chem. Abs.*, **133**, 368885.
1005. D. Yi, B. A. Ingelse, M. W. Duncan and G. A. Smythe, *J. Am. Soc. Mass Spectrom.*, **11**, 2000, 578.

2
Peptide Synthesis

BY DONALD T. ELMORE

1 Introduction

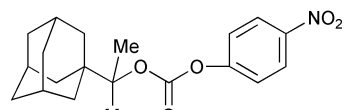
In contrast to the previous review,[1] there was a plethora of review articles in 2000 related to this subject. Some[2–12] cover various aspects of peptide synthesis whereas others are cognate to particular sections as follows: Section 2.1,[13–16] Section 2.2,[16] Section 2.3,[17] Section 2.4,[18] Section 2.5,[19–28] Section 2.6,[29–44] Section 2.7,[45–49] Section 3.1,[50–58] Section 3.3,[59–61] Section 3.4,[62–64] Section 3.5,[65–73] Section 3.6,[74–78] Section 3.8,[79–83] Section 3.9,[84–88] and Section 3.10.[89,90]

2 Methods

2.1 Amino-group Protection. – Removal of Boc groups from peptide derivatives containing Bu^t ester groups can be effected in good yield using either concentrated H_2SO_4 (1.5–3.0 equivalents) in $AcOBu^t$ or $MeSO_3H$ (1.5–3.0 equivalents) in $AcOBu^t/CH_2Cl_2$ (4:1 v/v).[91] *N*-Protected derivatives of β-amino acids are accessible from the corresponding derivative of an α-amino acid fluoride by the two-step Arndt–Eistert method of chain elongation.[92] The catalytic transfer hydrogenation method in presence of ammonium formate can be used to remove halogenated Z-groups.[93] Following a brief mention in the previous report,[1] the base-labile *N*-protecting group, 2-(4-nitrophenylsulfonyl)ethoxycarbonyl (Nsc) has been examined alongside the Fmoc group by two research teams.[94,95] The former team concentrated on the synthesis of melanotropins and analogues, but obtained yields that did not permit a clear distinction between the values of the Fmoc and Nsc groups. The other team found the Nsc group particularly suitable for SPPS when the amino acid derivatives are stored in solution and where loss of chiral purity is likely to occur during coupling. Clearly, the jury is still out and we await a more definite verdict. In addition, it would be helpful to compare the two groups in enzyme-catalysed syntheses. The Fmoc group can be removed efficiently without loss of chiral purity using $AlCl_3$ in toluene in solution syntheses,[96] but it is doubtful if this technique will gain much popularity. A catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in the presence of aliphatic or polymer-bound thiols removes Fmoc groups at room temperature.[97] Provided that the synthetic peptide does not contain disulfide bonds, this appears to be an

Amino Acids, Peptides and Proteins, Volume 33

attractive method. (2-Nitrofluorenyl)methoxycarbonyl amino acids can be prepared, perhaps surprisingly, by nitration of Fmoc amino acids with 100% HNO_3 in CH_2Cl_2.[98] It remains to be seen if this work offers more than a backward look to the sledgehammer chemistry of the 19th century. Chemistry that is much more in tune with the 21st century uses 2-(1-adamantyl)-2-propyl-(4′-nitrophenyl) carbonate (**1**) to prepare 2-(1-adamantyl)-2-propyloxycarbonyl amino acids.[99] The propargyloxycarbonyl group[100] can be introduced on to amino functions using propargyl chloroformate. The substituent is stable to neat CF_3CO_2H but is cleaved by a mixture of CF_3CO_2H and $Co_2(CO)_8$ in CH_2Cl_2. It is also removed by Zn/AcOH. The proposed abbreviation (PrOC) for this group could indicate other structures and Ppgoc is suggested as alternative. Another new protecting group for the amino function is the (2-phenyl-2-trimethylsilyl)ethoxycarbonyl substituent (Psoc)[101] that is removed under very mild conditions with Bu^n_4NF in CH_2Cl_2 more rapidly than is the 2-(trimethylsilyl)ethoxycarbonyl group leading to fewer side reactions.

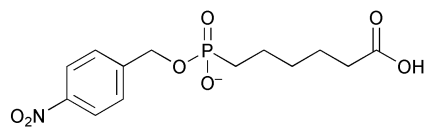


(1)

Finally, although not intended for peptide synthesis, the use of a bacterial deformylase enzyme to remove the *N*-formyl group of a 5′-formyldipeptidyl derivative of 5-fluorodeoxy-uridine (prodrug) that leads to the release of 5-fluorodeoxy-uridine[102] suggests that a revival of interest in the use of the formyl group in peptide synthesis may be on the cards.

2.2 Carboxy-group Protection. – α-Boc amino acids can be converted into α-amino acid methyl esters by reaction with Me_3SiCl/MeOH at room temperature overnight.[103] The Boc group is removed by the HCl that is generated during the esterification step. This method, however, is unlikely to replace the direct esterification of the parent amino acid. Pyridoxyl esters have been suggested for protecting carboxy groups because their multifunctional character allows further modification to be performed if the ester group is left attached at the end of the synthesis.[104] Amino acid derivatives can be esterified with (2-phenyl-2-trimethylsilyl)ethanol.[105] The esters readily undergo β-elimination with $Bu^n_4N^+F^-$ in CH_2Cl_2 to give styrene, Me_3SiF and the $Bu^n_4N^+$ salt of the carboxylic acid. The deprotection process is much more rapid than with the known (2-trimethylsilyl)ethyl group. If it is desired to esterify a peptide after SPPS on Kaiser oxime resin, this can be achieved while the peptide is still immobilised.[106] The peptidyl resin is left in MeOH or EtOH with Ca^{2+} or Eu^{3+} as catalyst. Does this reaction work with other primary alcohols such as benzyl alcohol? The nature of the *C*-terminal amino acid determines the rate of reaction. Similar exposure of the peptidyl resin to ammonia generates the amide. Deprotection of Bu^t esters can be effected with HNO_3 in CH_2Cl_2, but simultaneous oxidation of Tyr, Met and Trp limits the applicability of the method.[107] A case

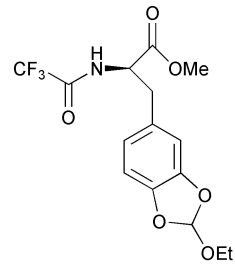
has been advanced[108] for using 4-hydroxyphenacyl esters because the protecting group can be removed by photolysis at 300 nm in water. The synthesis of such derivatives is achievable in good yield by reaction with 4-hydroxyphenacyl bromide in the presence of DBU. Removal of the protecting group is accompanied by rearrangement to yield 4-hydroxyphenylacetic acid. The 4-hydroxyphenacyl ester of bradykinin was synthesised and a single flash (<1 ns) liberated sufficient free peptide to activate bradykinin receptors as indicated by intracellular release of Ca^{2+} ions. A new route to quaternary Ser esters has been reported.[109] It will be interesting to learn the scope of this method. The 4-nitrobenzyl ester group has not achieved the popularity that one might have expected especially in view of the ease of synthesis of amino acid derivatives and of obtaining crystalline products. A new method of removing the 4-nitrobenzyl group uses as the catalyst an antibody (abzyme) raised with the hapten (**2**).[110] The antibody has been shown to catalyse the hydrolysis of monoesters of Glu. Again, one would like to know if this method or a development thereof is generally applicable to peptides.



(2)

2.3 Side-chain Protection. – Most of the new work in this area has been directed to protection of hydroxy groups. A method for the large-scale synthesis of *O*-Bu^t-serine has been described.[111] The propargyloxycarbonyl group mentioned above[100] has been used for protecting hydroxy groups. The method of Nishiyama and Kurita described last year for protecting the hydroxy group of Ser has been more fully described.[112] It has been shown to be applicable to the hydroxy group of Thr[112] and Tyr.[113] A potentially useful alternative uses $HO(CH_2)_3OH$ as a reagent to protect hydroxy groups.[114] This method possesses the useful property of increasing the water solubility of hydrophobic peptides. It was tested on the synthesis of a hydrophobic heptapeptide and more examples of its use are clearly desirable. After SPPS, the protected peptide can be detached using 1–5% CF_3CO_2H in CH_2Cl_2. The triethylene glycol moiety can then be removed with neat CF_3CO_2H without the occurrence of side reactions. The hydroxy function of Tyr can be blocked with the allyloxycarbonyl group.[115] This can be removed with good yields using 20% piperidine in $OHCNMe_2$. The synthesis of peptides of 3,4-dihydroxyphenylalanine (DOPA) offers a specific alternative for the protection of both hydroxy groups.[116] Reaction with ethyl orthoformate affords the cyclic acetal (**3**). Deprotection is effected by hydrolysis with NaOH in aqueous tetrahydrofuran. Using this approach, an adhesive peptide from mussel protein was synthesised.

In addition to its use to protect hydroxy groups, the cyclo-hexyloxycarbonyl group has been used to prevent the indole N atom of Trp from interfering in peptide synthesis.[117] Removal of the group, however, can be complicated by side reactions. Using HF in presence of anisole or 4-cresol, products involving

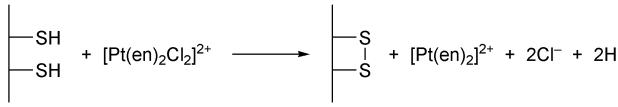


(3)

2,2′-coupling of two indole rings or similar coupling of one indole ring and an anisole molecule are formed. This side reaction can be prevented, however, by using Fmoc-Leu-OH as scavenger *without* the addition of cresol.

The problem of protecting thiol groups and their subsequent liberation, possibly accompanied by oxidation to the disulfide, still attracts attention. In the Fmoc SPPS procedure, 1M-Me_3SiBr in the company of PhSMe, CF_3CO_2H and $HS(CH_2)_2SH$ can be used to detach the peptide from the support and to remove all protecting groups on side chains.[118] Depending on the reagent used to protect the thiol group, the carbonium ion generated during the deprotection process can cause unwanted alkylation of the products. The amount of alkylation caused by various carbonium ions was compared.[119] It was found that the side reactions were within acceptable limits when using either the 2,4-dimethylpent-3-yl (Dmp) or 2,4-dimethylpent-3-yloxycarbonyl (Doc) groups and these are recommended as the best protecting groups for cysteinyl side chains. In a project that aimed to thiopalmitoylate a peptide while still on the resin, it was found that there can be problems if the Bu^{t}S group is used because the ease of deprotection depends on the nature of the peptide sequence.[120] It was recommended that better results are obtained if the 4-methyltrityl group is used. All is not necessarily satisfactory, however, when the 4-methyltrityl group is used to protect the side chain of Lys.[121] Selective deprotection of peptides containing additional trityl groups was monitored by chromatography and it was found that Trt groups were cleaved and some product was detached from the resin. Moreover, UV monitoring at 470 nm is regarded as inappropriate because there is insufficient difference between the molar extinctions of the two liberated cations.

2.4 Disulfide Bond Formation. – Endothelin-1 and its 1-16 fragment have been synthesised using H_2O_2 to oxidise the cysteinyl side chains to form the disulfide.[122] The authors claim that concomitant oxidation of Met is insignificant. The use of bispyridyl disulfide reported last year has been further studied.[123] After generation of an unsymmetrical disulfide by reaction of a cysteinyl peptide with 2,2′-bispyridyl disulfide $(Pys)_2$, the second stage involves reaction with another peptide that contains a cysteinyl residue. The reader is reminded, however, of the caveat in last year's report that better results were obtained using the more reactive 2,2′-bis(4-nitropyridyl) disulfide. An intriguing new method of forming intramolecular disulfide bonds in peptides uses *trans*-$[Pt(en)_2Cl_2]^{2+}Cl_2^-$ (Scheme 1).[124] Slightly acidic or neutral conditions are used and there is no oxidation of

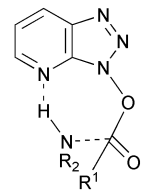


Scheme 1

Met residues. The kinetics are second order. The regiospecific production of disulfide bonds where more than one is present in the required product has provoked considerable interest. The use of a different protecting group for each pair of thiol groups to be oxidised is standard procedure and, in those cases where just two disulfide bonds are to be formed, the design of a one-pot protocol is usual. Thus, orexin A is a neuropeptide containing two disulfide bridges and these were generated in a one-pot method using Acm and Trt groups for orthogonal protection.[125] In the synthesis of conotoxin SI,[126] xanthen-9-yl (Xan) and Acm were chosen to provide orthogonal protection for pairs of thiol groups. Following SPPS of the linear protected precursors, The Xan groups were removed simultaneously with the detachment of the peptide from the polymer support. Production of the first disulfide bond was effected using either Me_2SO or a solid-phase version of Ellman's reagent. For generation of the second disulfide bond, $(CF_3CO_2)_3Tl$, I_2 or a sulfoxide/silyl mixture proved to be satisfactory. Alternatively, the same peptide was formed from the linear precursor using temperature-controlled removal of orthogonal protecting groups.[127] These were Bu^t and $CH_2C_6H_4Me$-4. The first pair of thiol groups were liberated and oxidised by treatment with $CF_3CO_2H/Me_2SO/PhOMe$ (97.9:2:0.1) at room temperature. Subsequent heating at 70 °C liberated the remaining thiol groups and, with probable aerial oxidation, this completed the synthesis. In another synthesis of endothelin-1, it was claimed that the presence of Trp in the peptide required the indole ring to be protected,[128] and the Doc group is preferred because of the mild conditions that sufficed for its removal. The orthogonal groups used were Acm and Bu^t for regioselective formation of the disulfide bridges. Insulin-like growth factor (somatomedin C) contains three disulfide bridges.[129] Synthesis by the recombinant DNA route gives four isomeric disulfides. Three contain the Cys^{18}–Cys^{61} bridge. The fourth does not contain this moiety and this peptide has been chemically synthesised.

2.5 Peptide Bond Formation. – Fmoc amino acid azides have been prepared from the corresponding acids and NaN_3 and either the unsymmetrical acid anhydride generated from Bu^iOCOCl or from the acid chloride.[130] The products were isolated as solids that were stable at room temperature. Some Fmoc dipeptide esters were made by coupling at room temperature during 18 h. Despite one short paper,[131] carbodiimides have lost their pole position to abundant and more modern reagents described later. When used in conjunction with either HOAt or HOBt, however, carbodiimides still have an important role. Methods for the preparation of *N*-protected amino acid fluorides and their application to the synthesis of small peptides have been described.[132,133] Aryl esters of *N*-protected amino acids have been prepared by Wolff rearrangement of α-*N*-protected α-diazoketones in the presence of suitable phenols.[134,135] The

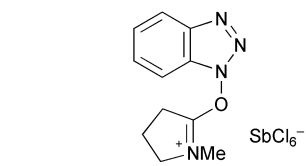
improvement of coupling yields obtained with aryl esters by addition of HOBt or HOAt has been confirmed.[136] It is now accepted that, although pentafluorophenyl esters give good results, other aryl esters are unlikely to supplant the newer coupling methods. The recent synthesis of 5-aza-1-hydroxybenzotriazole and the 6-aza-isomer has permitted a quantitative assessment of the relative values of all four isomers.[137] The best additive remains HOAt and Carpino *et al.* maintain that the possible existence of a seven-membered cyclic hydrogen bonded structure (**4**) with the electron-withdrawing effect of the pyridine nitrogen atom could explain the enhanced reactivity of the *O*-acyl ester. Recent



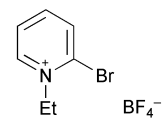
(4)

reviews of peptide synthesis may have given the impression that there are enough coupling methods and reagents to satisfy almost anybody. The flood of new reagents emanating from the Li and Xu laboratory in Shanghai, however, must appear daunting to a new entrant into the peptide field. Identification of these reagents by four- and five-letter abbreviations only compounds the difficulty of making a suitable choice. One could easily say that enough is enough, but what is now required is an agreed set of tests that will lead to a table of information about the preparation of a suite of peptides reporting important criteria such as yield, chiral purity, ease of isolation and purification. The suite of test peptides should include examples where there are known difficulties such as ease of loss of chiral purity, steric hindrance, adoption of unfavourable conformations by products, difficulty of removal of protecting groups and, where relevant, difficulty of detachment of product from macromolecular supports. The data accumulated should preferably reflect a consensus of results from several research groups. Available space does not allow description of all the new coupling agents that have been invented. Li and Xu have devoted much attention to immonium reagents. It was found that these reagents had a higher reactivity than did uronium compounds. Moreover, there was less loss of chiral purity when immonium reagents were used. It was argued that in uronium reagents, delocalisation of electrons can occur with both nitrogen atoms of the $>N–C–N<$ moiety resulting in a relatively high electron density at the central carbon atom. This is unfavourable to the nucleophilic attack by the carboxylate anion that is required to lead to the reactive intermediate that is involved in the formation of a peptide bond. This disadvantage is overcome by replacing one of the nitrogen atoms with hydrogen, alkyl or aryl. These immonium reagents can be prepared by treating *N*-disubstituted amides with bis(tri-chloromethyl) carbonate to give immonium chlorides. Treatment of these with $SbCl_5$ and then with potassium salts of active hydroxy compounds (*e.g.* HOBt) or the hydroxy compounds and

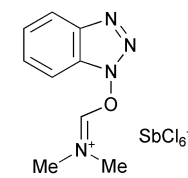
tertiary base afforded the desired reagents as stable solids. A few examples of such reagents are 5-(1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl-2*H*-pyrrolium hexachloroantimonate (BDMP, **5**), 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP, **6**) and (1*H*-benzotriazol-1-yloxy)-*N*,*N*-dimethylmethanimmonium hexachloro-antimonate (BOMI, **7**).[138–145] BEP was found to be particularly suitable for the synthesis of peptides of *N*-methylamino acids. BDMP was shown by X-ray crystallography to have the immonium group on N^3 of the benzotriazole ring rather than on the oxygen atom. It is possible that some of the other reagents may require revised structural formulae. An immobilised form of HOBt gives (**8**) on treatment with tetramethylchlorouronium tetrafluoroborate and this is described as being suitable for peptide coupling even in wet solvents.[146] Moreover, the immobilised HOBt can be recovered and recycled. Presumably, the analogous immobilised form of HOAt will soon be available. Another new coupling reagent (**9**) is formed from 2-chloro-4,6-dimethoxy[1,3,5] triazine and *N*-methylmorpholine and is recommended for SPPS.[147] *O*-(*N*-Succinimidyl)-1,1,3,3-tetramethyl-uronium tetrafluoroborate, *N*-hydroxysuccinimide and $CuCl_2$ is a mixture that is recommended for peptide bond formation free from loss of chiral purity especially where the *C*-terminal residue forming the peptide bond is an *N*-methylamino acid.[148,149] It is surprising that with recommendations for the use of $CuCl_2$ coming at intervals from different teams that its use is not more widespread.



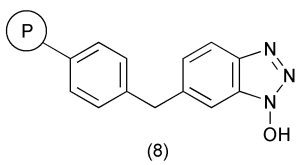
(5)



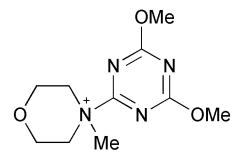
(6)



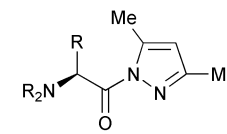
(7)



(8)

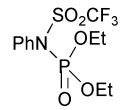


(9)

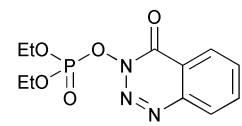


(10)

N-Acylpyrazoles (**10**) are readily prepared from amino acids and their derivatives and they react with amino acid esters to form peptide derivatives.[150] Fmoc amino acid esters have been used as the *C*-terminal component in the synthesis of dipeptide derivatives in a one-pot procedure.[151] The Fmoc group is removed by KF that is incorporated in the coupling mixture. The obvious snag with this method is that the Fmoc group cannot be used to protect the *N*-terminal residue. Also, the coupling step requires several hours and is unlikely to be acceptable despite the good yields obtained in these days where several steps are required to be accomplished in a working day. Phosphorylation of trifluoromethanesulfonanilide provided another coupling reagent (**11**) that proved successful in the synthesis of a highly hindered dipeptide, $ZNHCMe_2CONHCMe_2CO_2Me$.[152]



(11)

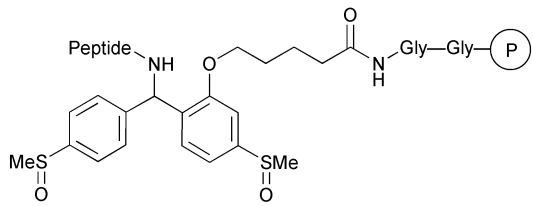


(12)

Tests on chiral purity of a model peptide in the Young racemisation test gave 2% racemisation. 3-(Diethoxyphosphoryloxy-1,2,3-benzo-triazin-4(3*H*)-one (DEPBT)(**12**) is reported to be very suitable for the synthesis of cyclopeptides.[153,154] Cyanuric fluoride is reported to afford better yields of sterically hindered peptides than does BOP-Cl.[155] 2-Pyridylthiol esters in presence of Me_3Al give good yields of peptide derivatives with good nucleophiles, but not with poor nucleophiles.[156] 4-Tolylthiol esters in presence of silver trifluoroacetate, in contrast, is less selective. Di(2-pyridyl) carbonate in the presence of a catalytic amount of DMAP gave good yields of various amides and a few simple peptides.[157] The method involves the intermediate formation of the 2-pyridyl esters of the *N*-protected amino acids.

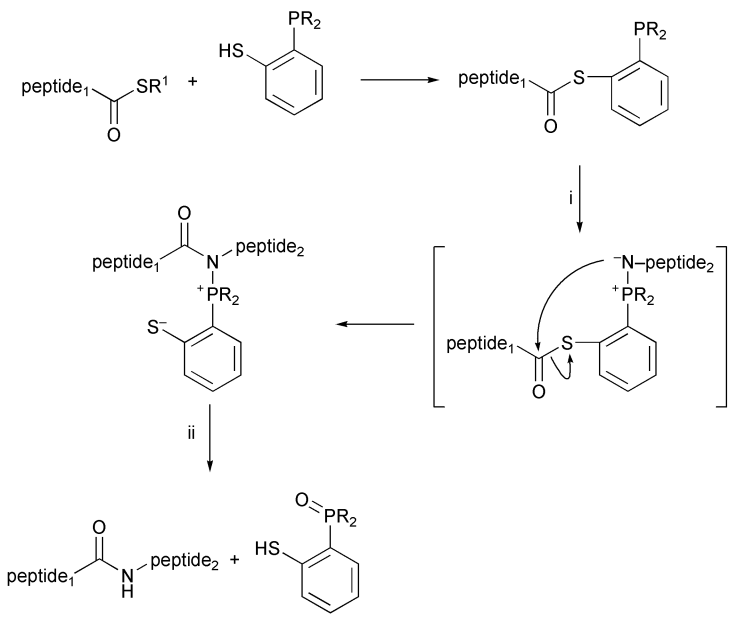
An interesting variation on the solution synthesis theme involves the use of an *N*-benzothiazole-2-sulfonyl (Bts) derivative of an amino acid.[158] These are easily obtained from Bts–Cl and can in turn be converted into the acyl chloride for coupling to another amino acid or peptide. The $-SO_2NH-$ moiety permits ready *N*-alkylation and the Bts group can be easily removed by three methods: (i) 50% H_3PO_2 slowly added to a refluxing solution in tetrahydrofuran, (ii) treatment with five equivalents of $NaBH_4$ near 0 °C, (iii) treatment with PhSH (2–3 equivalents) and K_2CO_3 (three equivalents). This last method usually gave the highest yield. It is sometimes refreshing to learn that peptide synthesis is not always plain sailing. Difficulties were encountered in the synthesis of peptides containing a Tic residue but were overcome using HOAt with either DIC or HATU.[159]

Native chemical ligation and related methods continue to attract adherents. A transcription factor that contains three zinc fingers, Zif 268, has been made by this method.[160] This technology can be combined with the biosynthetic production of an appropriate fragment.[161] The product ultimately obtained was a phosphoprotein. It has also been shown that expressed protein ligation can be carried out in the presence of a chemical denaturant such as guanidine hydrochloride permitting the introduction of amino acids that do not normally occur in proteins.[162] A different approach to the problem of introducing unnatural amino acids in the native chemical ligation method involves reaction of an amino group of one segment with thiolane-2,5-dione to produce a thioacid. This can be allowed to react directly with the amino group of a second peptide or can be converted into an intermediate benzyl ester by reaction with benzyl bromide before reaction with the second peptide.[163] If the first segment contains amino acids that contain appropriate side chains, it is possible to introduce two thiolesters groups to afford ultimately branched chain proteins. One can visualise such an approach being used to make polyenzymic assemblies for biotechnological purposes. Already, a possible methodology for the assembly of several peptide/protein fragments has been described.[164] It comprises the use of a solid-phase technique to simplify purification and a safety-catch linker that can be



(13)

simply made to be acid-labile at the appropriate time. The linker (**13**) was described by Patek and Lebl a decade ago. The sulfoxide groups render (**13**) stable to acid and acid hydrolysis is readily effected after reduction of the sulfoxide groups. The usual native chemical ligation method requires the presence of a cysteine residue at the *N*-terminus of one fragment, although Offer and Dawson have shown that the cysteine can be replaced by 2-mercaptobenzylamine.[165] This technique, however, leaves the aromatic moiety in the peptide chain. Perhaps the most ingenious advance in this field involves using the Staudinger reaction.[166] A phosphine is used to reduce an azide to an amine. An intermediate iminophosphorane, $R_3P^{+}-{}^{-}NR'$, is formed and the nucleophilic nitrogen can attack an acyl donor to form finally an amide. When applied to peptide synthesis, a phosphinothiol is used to effect the formation of the peptide bond (Scheme 2).



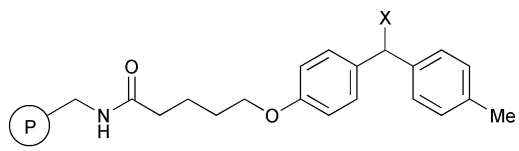
Reagents: i, N_3–peptide; ii, H_2O

Scheme 2

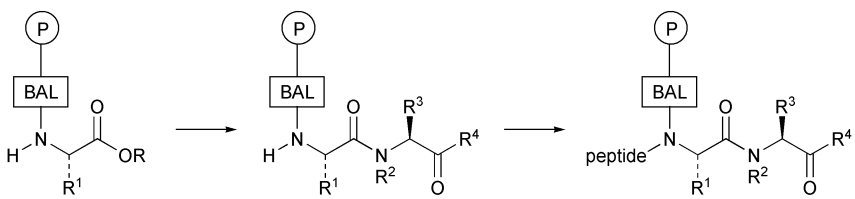
2.6 Peptide Synthesis on Macromolecular Supports and Methods of Combinatorial Synthesis. – There has been some activity with polystyrene-based supports, especially those having an open structure using, for example, alkanediol

methacrylate esters as crosslinking agents.[167–170] For example, ACP(33-42) was obtained easily in good yield that was better than when Merrifield resin was used.[168–170] A core-shell type of support with a polystyrene core and a PEG shell merits further study.[171] A chemically inert PEG-based resin was prepared by reductive amination of a mixture of mono- and dialdehyde PEG_{1500} and branched crosslinked tris(2-aminoethyl)amine.[172] The resin had excellent swelling properties and was stable to strong acids and bases. SPPS has been carried out on a chitosan/chitin support using three different linkers.[173] Various tetrapeptide derivatives were synthesised, including two that contained the difficult dipeptide sequence, -Aib-Aib-, as potential farnesyl transferase inhibitors. SPPS has also been conducted on cellulose membranes in order to produce peptide–cyanine dye conjugates as fluorescent contrast agents aimed at specific receptors of tumours.[174] For those who require an immobilised coupling reagent, BOP has been attached to Merrifield resin without the use of the carcinogenic $(Me_2N)_3PO$.[175]

The literature also contains the usual crop of new linkers. Replacement of the hydroxymethylphenoxyacetic acid linker with one containing a pentanoic acid chain is reported to result in increased loading capacity, yield and product purity.[176] The same group have described another linker containing a hydroxyethyl moiety that is labile to 20% CF_3CO_2H.[177] For the SPPS of peptide amides, an aminoethyl-polystyrene linker on a multipin support has also been described.[178] It is stable to mild acid treatment that cleaves Bu^t groups but 95% CF_3CO_2H detaches the peptide from the resin. Use of fluorinated linkers allows monitoring of cleavage of peptides from the support following direct study of the support on standard NMR equipment.[179] The 4-(4-methylphenylchloromethyl)phenoxy linker (**14**) is useful for the synthesis of pseudopeptides. If X = Cl, the resin linker conjugate can react with Fmoc derivatives of amino acids, amino alcohols and hydroxylamine.[180] Using a backbone amide linker (BAL),[181] a method (Scheme 3) has been reported for the synthesis of peptides with Pro, *N*-alkylaminoacyl or His at the *C*-terminus.[182] This procedure avoids the formation of diketo-piperazines and the loss of chiral purity. 3-Thiopropionic acid is a simple and versatile linker giving rise to a thioester link that

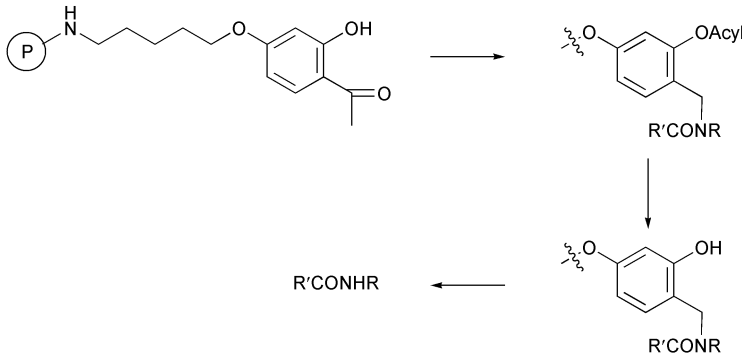


(14)

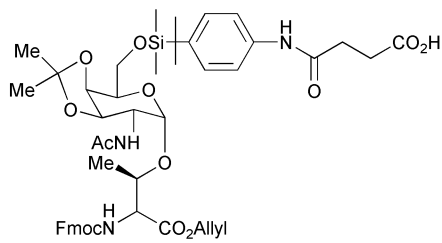


Scheme 3

can be cleaved with numerous nucleophiles to give *C*-terminally modified peptides.[183] A new linker provides access to peptides with a *C*-terminal thioacid.[184] A versatile aldehyde linker is useful for the SPPS (Scheme 4) of *C*-terminally



Scheme 4

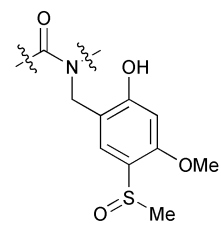


(15)

modified peptides.[185] Reductive amination involving the aldehyde group of the linker and an amine allows the construction of the peptide chain on the resultant secondary amine function. Temporary acylation of the aromatic hydroxy group confers stability to 95% CF_3CO_2H. After removal of the *O*-acyl group with 20% piperidine, the product could be detached with CF_3CO_2H. A new silyl linker (**15**) has been designed for the synthesis of glycopeptides.[186] The linker is attached to the resin by its carboxy group and to an *O*-glycoside of Fmoc-Ser-OH *via* the primary hydroxy group in the carbohydrate moiety. This permits chain elongation from either end of the Ser moiety. The peptide is detached from the support with F^- ion. Serglycin glycopeptides in which Ser and Gly occur alternately have been made using a new allyl ester linker. In another method for synthesising glycopeptides, t-butyl 6-bromo-(*E*)-hexenoate was caused to esterify an Fmoc amino acid yielding an allyl ester.[187] Removal of the Bu^t group then permitted attachment of the linker bearing the protected amino acid to acid-labile Sieber resin. The glycopeptide chain could now be constructed and, when complete, detached by Pd catalysis. A much more complicated application of a safety-catch linker involves the semisynthesis of vancomycin.[188] A polymer-bound phenyl seleno group functions as a pro-allyl saftey catch linker. The polymer-SeBr group can be alkylated using $LiBH_4$ and 3-iodopropanol. This provides a site of attachment for the vancomycin and after manipulations have been completed

the product is detached from the polymer using H_2O_2 followed by exposure to catalytic amounts of $[Pd(PPh_3)_4]$ and polymer-bound tin hydride.

There has been further work on the Johnson–Sheppard method of dealing with so-called difficult sequences. The use of the *N*-(2-hydroxy-4-methoxy benzyl) (Hmb) group is somewhat restricted by the limited incorporation into unhindered dipeptide sites. The problem is not related to incomplete *O*-acylation of the auxiliary Hmb group but rather to the poor $O \rightarrow N$ acyl transfer kinetics. It has been proposed[189] that the Hmb group should be replaced by the 2-hydroxy-6-nitrobenzyl (Hnb) group because of the enhanced rate of acyl transfer that is attributable to the presence of the electron-attracting nitro group. An added bonus is the improved cyclisation of small sterically constrained peptides. The Hnb group is introduced by reductive alkylation involving the *N*-terminal amino group of an intermediate peptide. The Hnb group is removed by photolysis (366 nm) in an orthogonal manner with respect to the Fmoc group. The phenolic group can be acylated with a protected amino acid and the Fmoc group can be removed with piperidine in the usual way. The completed peptide can be acidolytically detached from the support. Johnson has recognised the foregoing problem and has produced a slightly different solution.[190] He uses the 3-methylsulfinyl-4-methoxy-6-hydroxybenzyl group (**16**) (SiMB) and this is more more readily acylated than is the case with the Hmb group. After completion of the peptide assembly, reduction of the sulfoxide group confers acid lability on the 3-methylthio-4-methoxy-6-hydroxybenzyl moiety, a property that was used in the application of the Msob group. There is a minor reservation concerning the abbreviation used for this method. To the uninitiated, SiMB might suggest a linker that would be sensitive to F^-; MSMHB is only one character longer and might be more easily recognised.



(16)

Two methods have been described that permit the use of Fmoc chemistry in the preparation of protected peptide thioesters required for native chemical ligation. In one case,[191] SPPS is used to assemble a peptide using Fmoc chemistry and the thioester is generated when the peptide is detached from the resin using Me_2AlCl and EtSH. Unfortunately, there is a tendency for some loss of chiral purity and for the formation of some oxyester. The other method,[192] uses more conventional methodology but uses DBU to remove the Fmoc group.

SPPS can be conducted using α-azido acid chlorides.[193] This avoids the need for choosing an appropriate protecting group for the α-amino group of an amino acid. Side reactions have not been reported and chiral purity is maintained. *C*-Terminal peptide aldehydes are of considerable interest as potential pro-

teinase inhibitors and there has been considerable effort applied to their synthesis.[33,194] A favoured route starts from an amino acetal conjugated to the support through a backbone amide linker.

We come now to a section devoted to problems and useful tips in SPPS. Although cyclopeptides are not discussed in detail in this chapter, it is pertinent to mention that attempted synthesis of cyclic peptides formed by linking the side chains of lysine and glutamic acid did not proceed according to plan when the Dmab group was used to protect Glu.[195] α-*N*-Pyroglutamyl chain-terminated peptides were formed during the synthesis of fully protected targets. This behaviour was not found when the γ-carboxyl group of Glu was protected with Bu^t. The attempted synthesis of Asn peptides can give rise to unwanted byproducts such as aspartimides, a mixture of α and β-peptides and piperidides if piperidine is used in peptide release.[196] This problem can be circumvented in SPPS/Boc by using *N*-[(hydroxy-methyl)-2-fluorenyl]succinamic acid (HMFS) as the linker since this permits the use of the less basic morpholine for peptide release without the risk of the formation of morpholides or αβ isomerisation. For the same problem, the use of piperazine containing 0.1 M HOBt has been recommended.[197] A more exhaustive comparative examination seems desirable. The formation of deletion peptides can occur in SPPS/Boc especially at *N*-terminal His(Bom) due to incomplete removal of the Boc group even with quite high concentrations of CF_3CO_2H.[198] Longer exposure times to acid and/or the use of more concentrated CF_3CO_2H is recommended. Periodic analysis of a cleaved sample by hplc could be an additional safeguard. Photolytic cleavage during SPPS can cause problems.[199] Thiol groups, if present, can be a target for trouble, but the full extent of possible complications appears not to have been unravelled. Obviously, if an enzyme is to be used, during the synthesis, it should not be present before photolysis has been completed and any oxidising species arising therefrom have been removed. The synthesis of azapeptides by SPPS is prone to capping by ring-closure of an *N*-terminal azaamino acid residue to form a hydantoin.[200] This problem can be obviated by temporary protection with $Fmoc_2$(Hmb)XaaOPfp where Xaa is the terminal residue before attachment of the azaamino acid residue. Previous difficulties in the synthesis of Arg peptides have led to a long search for a suitable protecting group for the guanidino function and the Pmc group is generally regarded as the most suitable function. Synthetic routes that rely on the protonation of the guanidino group have been tried in the past but the risk of lactamisation involving the guanidino group has tended to dissuade researchers from using this route. Nevertheless, there is another claim that protonation of the side chain of Arg is sufficient to exclude this possibility.[201] The reader is reminded of the possibility of synthesising an Orn peptide and guanidinating this after all amino acids have been assembled in the desired peptide (see ref. 1, Section 2.8). This method has now been applied to the synthesis of tetra-substituted derivatives (including peptides) of lysine.[202] Peptides of Cys cannot be assembled satisfactorily by the SPPS/Fmoc procedure using Cys bearing either the Pys or Npys groups. If one of these groups is required, it is recommended that the required peptide sequence is assembled using Trt protection on the thiol group(s). During acid-catalysed detachment of

the product from the resin, incorporation of 2,2′-dithio-bis(pyridine) blocks the thiol group(s) since disulfide exchange occurs under acidic conditions.[203]

There have been some other advances that merit mention in this section. The use of a 'trimethyl lock' in a safety-catch linker, mentioned before (see ref. 1, Section 2.6), has received further support.[204] Capping of an analytical sample of resin with chloroacetyl chloride followed by reaction with ammonia at intervals during sequence assembly permits application of the Edman method of stepwise degradation to confirm that no omissions or side reactions have occurred.[205] A combination of SPPS and solution-phase synthesis has been recommended.[206] Boc/Bzl chemistry and a base-labile linker, *N*-[9-(hydroxymethyl)-2-fluorenyl] succinamic acid were used. The 2-BrZ group for Tyr and HCO- group for Trp were not used because of their base-lability. The anchoring group was cleaved with morpholine or piperidine and the assembled fragments were coupled in solution. Although peptide synthesis from the *N*-terminus to the *C*-terminus has been largely eschewed from early days because of the risk of extensive loss of chiral purity, two papers invite us to think again. One method[207] uses 2-chlorotrityl resin with allyl ester for temporary protection and the use of the Cu complex of HOBt during coupling with either diisopropyl carbodiimide or HATU. With the former coupling method, only 0.6% of enantiomerisation occurred but less satisfactory results were obtained with HATU. The other approach[208,209] involves reacting 4-nitrophenyl chloroformate with Wang resin and then attaching the *N*-terminal residue as its Li salt in $OHCNMe_2$. The immobilised amino acid was treated with HBTU and hydroxysuccinimide for 30 min. before addition of the next amino acid as its Li salt. Although no loss of chiral purity was detected, the methods used were not particularly sensitive so further examination of this procedure is desirable. Traceless SPPS of peptides containing 3-aryl β-amino acids uses a silylated side chain.[210] This procedure allowed the synthesis of cyclic peptides.

A few papers have appeared describing peptide libraries. A library of 62 000 peptides has been assembled that can recognise a monosaccharide specifically using both sandwiching and hydrogen-bonding interactions.[211] Using cholic acid as a skeleton on which to hang peptides made by combinatorial synthesis, pendant groups bearing aminosubstituents were attached at positions 3 and 12.[212] Small peptides containing His and Ser were then assembled by combinatorial synthesis on to these amino groups and some of the products displayed hydrolytic activity towards 4-nitrophenyl esters and were considered to be models of the serine proteinases. A cyclic pseudotripeptide platform has been synthesised as a platform on which amino groups can be attached at the three carboxy groups members of a peptide library.[213] Finally, polyoxyethylene–polyoxypropylene resin was derivatised with a 4-hydroxymethylphenoxy linker and used as a solid support for aldol reactions that are catalysed by yttrium or ytterbium triflate in aqueous solution.[214] A peptide bearing an aldehyde moiety at the *N*-terminus is attached at its *C*-terminal carboxy group.

2.7 Enzyme-mediated Synthesis and Semisynthesis. – Publications on this topic usually include information on several aspects of the topic such as choice of

enzyme including nonproteolytic enzymes, physical state of enzyme used as catalyst, choice of medium for synthesis, structure of substrate(s), use of enzymes for deprotection. Space does not allow detailed information on these matters for each study reported. Papers mentioned have been selected in the main for one reason but readers of the original literature are very likely to find several points of interest in each paper.

Enzymes are frequently immobilised[215–218] and are then often used in neat organic solvents when high yields are the norm.[218] Even when the enzyme is in the free state but used in a medium in which it does not dissolve, the past history of the enzyme can be important. For example, using thermolysin as the catalyst in MeCN as the reaction medium, the highest rate of synthesis was obtained when the enzyme was added in aqueous suspension, but was greatly diminished when the enzyme was added as a freeze-dried powder.[219] The use of enzyme encapsulated in reversed micelles is still occasionally used as instanced with α-chymotrypsin in micelles formed with $C_{14}H_{29}NMe_3^+Br^-$ and heptane/octanol.[220] In $OHCNMe_2$/MeCN containing 6% water, α-chymotrypsin was inactive when the concentration of $OHCNMe_2$ was $>60\%$, but when the enzyme was noncovalently complexed with polyacrylate, it was very effective under such conditions.[221] Ac-Tyr-Lys-NH_2 was obtained in 95% yield. Complexes of enzymes and nonionic detergents have remarkably increased activity for peptide synthesis by comparison with the freeze-dried enzyme. Addition of an organic solvent can further enhance the activity.[222] Zeolites are useful supports for some proteolytic enzymes.[223]

An interesting observation was made when *Staphylococcus aureus* V8 proteinase was used in aqueous solution. A sequence from RNase A, TAAAKFE, formed oligomers at pH 6–8 and 4 °C; replacement of any residue by Gly interfered with this process.[224] Details have been reported of the synthesis of small peptides catalysed by trypsin,[225,226] thermolysin,[227–229] pseudolysin,[230] subtilisin[231] and *Pseudomonas aeruginosa* proteinase.[232,233] Dipeptides that are potential intermediates for the production of aspartame are popular targets.[216,229,234,235] There is a further report on the use of supercritical CO_2 as a highly suitable medium for enzyme-catalysed synthesis.[236] A linear free energy relationship with respect to solvent polarity has been established.[237] The intrinsic activation energy for subtilisin catalysis is lowest in polar organic solvents possibly due to the stabilisation of the transition state for the transesterification step. The impressive results obtained in the the so-called solid phase coupling method has produced only two publications.[219,238]

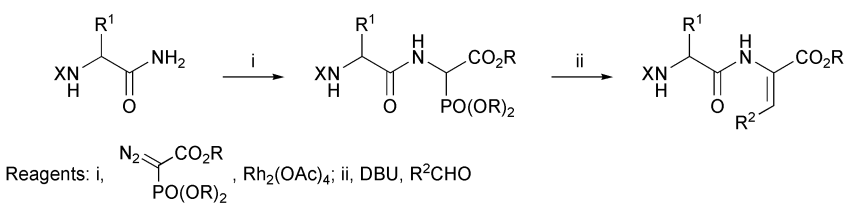
Peptide synthesis in frozen solution remains an attractive area for study. Coupling of Ac-Phe-OEt and H-Ala-NH_2 using α-chymotrypsin in aqueous solution at 25 °C gave $\leq 21\%$ whereas in frozen solution at -30 °C a 60% yield of peptide was obtained.[239] The same peptide was synthesised from Ac-Phe-OH and again the yields in frozen solution were better than in liquid solution.[240] The Glu/Asp endopeptidase from *Bacillus licheniformis* accepts amino components for peptide synthesis in frozen solution that do not react at room temperature.[241] The same researchers suggest that the reverse reaction, proteolysis, is suppressed in frozen solution thereby leading to improved yields.[242] Not only are yields

improved in frozen solution, but substrate specificity is broadened.[243] These effects are not due only to the lower temperature, since addition of $<1\%$ of polyethylene glycol suppresses the broader S′-specificity found in frozen solution.[244] It is postulated that interactions between enzyme and ice structure are important. It would be interesting to examine the effect of freezing a solution of enzyme in D_2O on the course and kinetics of peptide coupling.

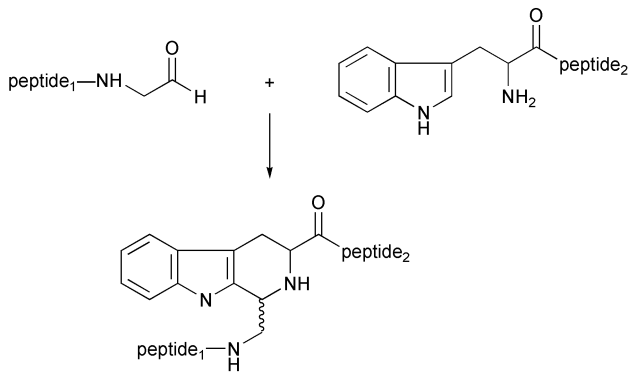
The specificity of proteolytic enzymes that undergo an intermediate esterification step can be broadened by using esters that are more reactive towards nucleophiles. Carbamoylmethyl esters, for example, are suitable substrates.[245–248] The well-known tactic of using inverse substrates, that is substrates with a positively charged ester group but with an uncharged side chain, with trypsin or clostripain has been further exploited.[249–251] This should not be regarded as simply an enzymic curio; once the positively charged alkyl group has departed and coupling has occurred, the product is no longer attacked by an enzyme such as trypsin. Consequently, reaction reversal is impossible. Some compounds with a positively charged ester group can also function as substrates for chymotrypsin.[252]

The apparently limitless prospect of using genetically engineered proteolytic enzymes for peptide synthesis surprisingly has received little attention to date. A double mutant of subtilisin E (K170C, E195C) forms a disulfide bridge where presumably ion-pairing occurred in the native enzyme and this resulted in a decrease in k_{cat}.[253] Replacement of the active site residue, Ser^{195}, by either Ala or Gly produces a mutant with diminished hydrolytic activity but which still mediates peptide bond formation presumably through an acyl enzyme involving His^{57}.[254] The use of enzymes to deprotect derivatives of synthetic peptides has similarly not attracted much attention. One wonders if a few synthetic chemists still regard enzymes as figments of the biochemist's imagination. Penicillin G acylase has again been used to remove the [4-(phenylacetyl)oxy]benzyloxycarbonyl group (PhAcOZ) during the synthesis of a biotin-labelled glycophosphononapeptide from the *c*-Myc oncoprotein.[255] Tetra-*O*-acetyl-D-glucopyranosyloxycarbonyl- and tetra-*O*-acetyl-D-galactopyranosyloxycarbonyl- groups have been developed and used as protecting groups in the synthesis of dipeptides.[256] It was shown that the latter group could be removed by a two-step enzymic process using (a) lipase to remove acetyl groups, (b) β-galactosidase and this allows spontaneous fragmentation of the remainder of the protecting group. Enzymic enantioselectivity of lipase in organic solvents can be substantially increased by adding chiral agents that form salts with the substrate. For example, propanolysis of Phe–OMe in dry MeCN catalysed by *Pseudomonas caparia* lipase affords a $(k_{cat}/K_m)_S/(k_{cat}/K_m)_R$ of 5.8. Addition of (*R*)-10-camphorsulphonic acid, however, increases this to 53.4.[257] A cell-free extract of *Streptomyces albulus* (KO-23) catalyses the conversion of diketopiperazines that derived from Phe and an aliphatic amino acid into dehydro derivatives.[258] Tetradehydro derivatives inhibit cell division in the sea urchin embryo. Finally, a 23-residue fragment of the PKA-anchoring protein (Ht31) from human thyroid has been made by semisynthesis using α-chymotrypsin-catalysed segment condensation.[259]

2.8 Miscellaneous Reactions Related to Peptide Synthesis. – Iodination of peptides and proteins is still of considerable interest for radioimmunoassays. By using the IPy_2BF_4 reagent in acidic medium, iodination is limited to the production of mono-iodotyrosine residues.[260] [^{125}I]-*N*-succinimidyl-4-iodobenzoate has been developed as a reagent particularly recommended for labelling monoclonal antibodies.[261] An ingenious method for labelling proteins in lysosomes has been developed.[262] These cells internalise positively charged molecules such as D-Lys-D-Arg-D-Tyr-D-Arg-D-Arg. This can be labelled with ^{125}I and then coupled to appropriate monoclonal antibodies. The carrier peptide will of course resist proteolysis. Past monarchs of the peptide field probably never considered ordering a bottle of samarium iodide. They missed an interesting trick. Small Gly peptides can be brominated with NBS on the Gly -CH_2- group and the product on successive reaction with 2-mercaptopyridine and SaI_2 at room temperature in presence of alkyl aldehydes and ketones acquires carbinol side chains on the Gly residues.[263,264] The authors suggest that this offers a new route to peptide libraries. In contrast, *N*,*C*-protected peptides of Gly undergo oxidation to α-ketoamides when treated with peracetic acid in presence of $RuCl_3.nH_2O$.[265] An intriguing method for the synthesis of dehydropeptides again requires resource to a catalogue of rather rare inorganic compounds.[266] Instead of being involved with the generation of a peptide bond, this method involves an N–H bond insertion procedure outlined in Scheme 5. The catalyst is $Rh_2(OAc)_4$. The N–H insertion product is used directly in the Wadsworth–Emmons reaction with an aldehyde or ketone. Clearly, advanced inorganic chemistry is a valuable source of catalysts for aspiring peptide chemists. A peptide with a *C*-terminal aldehyde group and a second peptide with an *N*-terminal Trp residue undergo a Pictet–Spengler reaction in acetic acid solution (Scheme 6).[267] Since the addition of a Trp residue at the *N*-terminus of a carrier protein would be simple, this might offer a valuable method for attaching an epitopic peptide for antibody production. Finally, during the attempted synthesis of a 61-residue peptide containing a Met residue in each fragment, an unexpected byproduct was formed in which one fragment had reacted with the other with the formation of an *S*-sulfonium derivative.[268] The reaction was apparently specific since only one Met residue was modified.



Scheme 5



Scheme 6

3 Appendix: A List of Syntheses in 2000

The syntheses in Section 3.1 are listed under the name of the peptide/protein to which they relate, but no arrangement is attempted under the subheading. In some cases, closely related peptides are listed together.

Peptide/Protein *Ref.*

3.1 Natural Peptides, Proteins and Partial Sequences. –

Aeruginosin
- Total synthesis and reassignment of configuration 269

Amamistatin
- Total synthesis of amamistatin A 270

Amyloid peptides
- β-Hairpin region of OspA single-layer β-sheet 271
- Solution synthesis 272
- SPPS of proteins associated with neurodegenerative disease 273

Angiotensin
- Cyclic analogue of angiotensin II 274

Antibiotics
- Picolyl derivatives of gramicidin S 275
- Analogues of gramicidin S 276
- Aib analogues of gramicidin B containing *C*-terminal Trp 277
- Putative intermediate in vancomycin biosynthesis 278
- Deglycobleomycin A_5 279
- Bis(polyamides) related to distamycin 280
- Bactonecin 5 model peptides rich in Arg and Pro 281
- Protegrin analogues containing up to 3 -SS- bonds 282
- Human defensins and analogues 283
- Models of antibacterial fragments of chromogranin A 284
- Distamycin analogues 285, 286

4 References

1. D.T. Elmore, *Specialist Periodical Report*: *Amino Acids*, *Peptides and Proteins*, 1999, **32**, pp. 107–162.
2. J.A. Borgia and G.B. Fields, *Trends Biotechnol.*, 2000, **18**, 243.

3. L.P. Miranda and P.F. Alewood, *Biopolymers*, 2000, **55**, 217.
4. K. Rose, *Protein Eng. Semisynth.*, 2000, 25.
5. S. Sakakibara, *Collect. Symp. Ser.*, 1999, **1**, 1.
6. G.J. Cotton, M.C. Pietanza and T.W. Muir, *Drugs Pharm. Sci.*, 2000, **101**, 171.
7. B.A. Moss, *Pharm. Formulation Dev. Pept. Proteins*, 2000, 1.
8. K.H. Mayo, *Trends Biotechnol.*, 2000, **18**, 212.
9. L. Andersson, L. Blomberg, M. Flegel, L. Lepsa, B. Nilsson and M. Verlander, *Biopolymers*, 2000, **55**, 227.
10. C.J.A. Wallace, *Protein Eng. Semisynth.*, 2000, 1.
11. C.H. Hassall, *Collect. Symp. Ser.*, 1999, **1**, 170.
12. A. Otaka, *Yakugaku Zasshi*, 2000, **120**, 54.
13. J.M. Brass, J. Frank, F.W. Wagner, H. Stocker and E. Wunsch, *Collect. Symp. Ser.*, 1999, **1**, 165.
14. G.C. Barrett, in 'Practical Approach in Chemistry: Amino Acid Derivatives', ed. G.C. Barrett, Oxford University Press, 1999, p. 7.
15. G.C. Barrett, in 'Practical Approach in Chemistry: Amino Acid Derivatives', ed. G.C. Barrett, Oxford University Press, 1999, p. 21.
16. F. Albericio, *Biopolymers*, 2000, **55**, 123.
17. V. Figala, E. Sturm, W.-R. Ulrich, K. Klemm and M. Przybylski, *Collect. Symp. Ser.*, 1999, **1**, 159.
18. K. Akaji, *Seitai Bunshi Kaiseki Kenkyu Senta Dayori*, 2000, **21**, 29.
19. P.E. Dawson and S.B.H. Kent, *Annu. Rev. Biochem.*, 2000, **69**, 923.
20. J.P. Tam, Q. Yu and Z. Miao, *Biopolymers*, 2000, **51**, 311.
21. D.M. Coltart, *Tetrahedron*, 2000, **56**, 3449.
22. P. Clapes, G. Caminal, J.A. Feliu and J. Lopez-Santin, in 'Methods in Non-aqueous Enzymology', ed. M.N. Gupta, Birkhaeuser Verlag, Basle, 2000, pp. 110–132.
23. P. Li and J.C. Xu, *Tetrahedron*, 2000, **56**, 9949.
24. Z.J. Kaminski, *Biopolymers*, 2000, **55**, 140.
25. B. Wang, S. Gangwar, G. Pauletti, T. Siahaan and R.T. Borchardt, *Methods Mol. Med.*, 2000, **23**, 53.
26. S. Gangwar, G.M. Pauletti, T.J. Siahaan, V.J. Stella and R.T. Borchardt, *Methods Mol. Med.*, 2000, **23**, 37.
27. Y.-C. Tang, G.-L. Tian and Y.-H. Ye, *Gaodeng Xuexiao Huaxue Xuebao*, 2000, **21**, 1056.
28. T.J. Deming, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 3011.
29. R.C. Sheppard, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford Univ. Press, Oxford, 2000, p. 1.
30. P.D. White and W.C. Chan, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford Univ. Press, Oxford, 2000, p. 9.
31. F. Albericio, I. Annis, M. Royo and G. Barany, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p. 77.
32. M. Quibell and T. Johnson, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p. 115.
33. S.L. Mellor, D.A. Wellings, J.-A. Fehrentz, M. Paris, J. Martinez, N.J. Ede, A.M. Bray, D.J. Evans and G.B. Bloomberg, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p. 137.
34. K. Barlos and D. Gatos, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p. 215.
35. J.W. Drijfhout and P. Hoogerhout, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p. 229.

36. J.P. Tam and Y.-A. Lu, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p. 243.
37. P. Mascagni, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p. 265.
38. B. Dorner, J.M. Ostresh, R.A. Houghten, R. Frank, A. Tiepold, J.E. Fox, A.M. Bray, N.J. Ede, I.W. James and G. Wickham, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, Oxford, 2000, p. 303.
39. L.E. Cammish and S.A. Kates, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, Oxford, 2000, p. 277.
40. M. Ruvo and G. Fassina, *Comb. Chem. Technol.*, 1999, 7.
41. A.F. Spatola and P. Romanovskis, in 'Amide Linkage,' eds. A. Greenberg, C.M. Breneman and J.F. Liebman, J. Wiley and Sons, Inc., New York, 2000, p. 519.
42. H.A. Remmer and G.B. Fields, *Drugs Pharm. Sci.*, 2000, **101**, 133.
43. B. Merrifield, *Collect. Symp. Ser.*, 1999, **1**, 12.
44. V.J. Hruby, *Collect. Symp. Ser.*, 1999, **1**, 34.
45. D. Ullmann and H.-D. Jakubke, in 'Proteolytic Enzymes,' eds. E.E. Sterchi and W. Stoeker, Springer, Berlin, 1999, p. 312.
46. H.-D. Jakubke, *Collect. Symp. Ser.*, 1999, **1**, 47.
47. D.B. Smithrud, P.A. Benkovic, S.J. Benkovic, V. Roberts, J. Liu, I. Neagu, S. Iwama, B.W. Phillips, A.B. Smith and R. Hirschmann, *Proc. Natl. Acad. Sci., U.S.A.*, 2000, **97**, 1953.
48. H. Noritomi, *Methods Biotechnol.*, 2000, **13**, 189.
49. P.A. Bentley, in 'Biotechnology' (2nd ed.), ed. D.R. Kelly, Wiley-VCH Verlag GmbH, Weinheim, 2000, pp. 491–517.
50. J.G. Davies and C.J.A. Wallace, in 'Protein Engineering and Semisynthesis,' ed. C.J.A. Wallace, C.R.C. Press, Boca Raton, FL, 2000, p. 73.
51. M.P. Dwyer and S.F. Martin, *Methods Mol. Med.*, 1999, **23**, 407.
52. B. Wang, D. Shan, W. Wang, H. Zhang, O. Gudmundsson and R.T. Borchardt, *Methods Mol. Med.*, 2000, **23**, 71.
53. P.J. Gilligan, D.W. Robertson and R. Zaczek, *J. Med. Chem.*, 2000, **43**, 1641.
54. M. Rautenbach and J.W. Hastings, *Chim. Oggi.*, 1999, **17**, 81.
55. I. Clark-Lewis, *Methods Mol. Biol.*, 2000, **138**, 47.
56. F. Yokokawa and T. Shioiri, *Yuki Gosei Kagaku Kyokaishi*, 2000, **58**, 634.
57. R.-H. Mattern, S.B. Moore, T.-A. Tran, J.K. Rueter and M. Goodman, *Tetrahedron*, 2000, **56**, 9819.
58. J.-M. Sabatier, *Anim. Toxins*, 2000, 196.
59. O. Melnyk, J.-A. Fehrentz, J. Martinez and H. Gras-Masse, *Biopolymers*, 2000, **55**, 165.
60. F. Bordusa, *Braz. J. Med. Biol. Res.* 2000, **33**, 469.
61. M.C. Hillier and S.F. Martin, *Methods Mol. Med.*, 1999, **23**, 397.
62. J. Zabrocki and G.R. Marshall, *Methods Mol. Med.*, 1999, **23**, 417.
63. H. Kessler, R. Konat, M. Kurz and C. Gilon, *Collect. Symp. Ser.*, 1999, **1**, 86.
64. L. Halab, F. Gosselin and W.D. Lubell, *Biopolymers*, 2000, **55**, 101.
65. J. Kihlberg, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p. 195.
66. R.R. Schmidt and K.-H. Jung, *Carbohydr. Chem. Biol.*, 2000, **1**, 5.
67. H. Hojo and Y. Nakahara, *Curr. Protein Pept. Sci.*, 2000, **1**, 23.
68. H. Kunz, B. Lohr and J. Habermann, in 'Carbohydrates', ed. P. Finch, Kluwer Academic Publishers, 1999, p. 187.
69. P.M. St. Hilaire and M. Meldal, *Angew. Chem., Int. Ed.*, 2000, **39**, 1163.

70. O. Seitz, *ChemBioChem*., 2000, **1**, 214.
71. H. Kunz and M. Schultz, *Carbohydr. Chem. Biol*., 2000, **1**, 267.
72. H.P. Wessel, *Carbohydr. Chem. Biol*., 2000, **1**, 565.
73. H. Herzner, T. Reipen, M. Schultz and H. Kunz, *Chem. Rev*., 2000, **100**, 4495.
74. P.D. White, in 'Fmoc Solid Phase Peptide Synthesis', eds. W.C. Chan and P.D. White, Oxford University Press, 2000, p.183.
75. S.V. Galushko, in Aminophosphonic Aminophosphinic Acids, eds. V.P. Kukhar and H.R. Hudson, John Wiley and Sons, Ltd., Chichester, 2000, p. 205.
76. P. Kafarski and B. Lejczak, in Aminophosphonic Amino- phosphinic Acids, eds. V.P. Kukhar and H.R. Hudson, John Wiley and Sons, Ltd., Chichester, 2000, p. 173.
77. P. Kafarski and B. Lejczak, in 'Aminophosphonic Amino- phosphinic Acids', eds. V.P. Kukhar and H.R. Hudson, John Wiley and Sons, Chichester, 2000, p. 407.
78. D.St.C. Green, E. Skordalakes, M.F. Scully and J.J. Deadman, in 'Aminophosphonic Aminophosphinic Acids', eds. V.P. Kukhar and H.R. Hudson, John Wiley and Sons, Chichester, 2000, p.570.
79. A. Ray and B. Norden, *FASEB J*., 2000, **14**, 1041.
80. S. Jordan and C. Schwemler, *Bioorg. Chem*., 1999, 262.
81. C.-H. Tung and S. Stein, *Bioconjugate Chem*., 2000, **11**, 605.
82. P.E. Neilsen, in 'Pharm. Aspects Oligonucleotides,' eds. P. Couvreur and C. Malvy, Taylor & Francis Ltd., London, 2000, p. 98.
83. D.A. Stetsenko, A.A. Arzumanov, V.A. Korshun and M.J. Gait, *Mol. Biol*., 2000, **34**, 852.
84. K. Luthman, S. Borg and U. Hacksell, *Methods Mol. Med*., 2000, **23**, 1.
85. M. Taddei, *Methods Mol. Med*., 1999, **23**, 189.
86. D.S. Perlow and R.M. Freidinger, *Methods Mol. Med*., 1999, **23**, 209.
87. W.M. Kazmierski and P.C. Fritch, *Methods Mol. Med*., 1999, **23**, 281.
88. L. Chaloin, M.C. Morris, N. Van Mau, J. Mery, G. Divita and F. Heitz, *Curr. Top. Pept. Protein Res*., 1999, **3**, 153.
89. U.J. Nilsson, *J. Chromatogr. A*, 2000, **885**, 305.
90. L. Schweitz, M. Petersson, T. Johansson and S. Nilsson, *J. Chromatogr., A*, 2000, **892**, 203.
91. L.S. Lin, T. Lanza, S.E. de Laszlo, Q. Truong, T. Kamenecka and W.K. Hagmann, *Tetrahedron Lett*., 2000, **41**, 7013.
92. K. Ananda, H.N. Gopi and V.V.S. Babu, *J. Pept. Res*., 2000, **55**, 289.
93. D.C. Gowda, B. Rajesh and S. Gowda, *Indian J. Chem*., 2000, **39B**, 504.
94. P.M. Balse, H.J. Kim, G. Han and V.J. Hruby, *J. Pept. Res*., 2000, **56**, 70.
95. C. Carreno, M.E. Mendez, Y.-D. Kim, H.-J. Kim, S.A. Kates, D. Andreu and F. Albericio, *J. Pept. Res*., 2000, **56**, 63.
96. A. Leggio, A. Liguori, A. Napoli, C. Siciliano and G. Sindona, *Eur. J. Org. Chem*., 2000, 573.
97. J.E. Sheppeck, H. Kar and H. Hong, *Tetrahedron Lett*., 2000, **41**, 5329.
98. B. Henkel and E. Bayer, *Synthesis*, 2000, 1211.
99. J.R. McClure and M.K. Sieber, *Heteroat. Chem*., 2000, **11**, 192.
100. Y. Fukase, K. Fukase and S. Kusumoto, *Pept. Sci*., 1999, 93.
101. M. Wagner, S. Heiner and H. Kunz, *Synlett*, 2000, 1753.
102. Y. Wei and D. Pei, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1073.
103. B.-C. Chen, A.P. Skoumbourdis, P. Guo, M.S. Bednarz, O.R. Kocy, J.E. Sundeen and G.D. Vite, *J. Org. Chem*., 1999, **64**, 9294.
104. L.Yu. Sklyarov, I.N. Sbitneva, N.A. Kopina and I.G. Sidorovich, *Russ. J. Bioorg.*

Chem., 2000, **26**, 245.
105. M. Wagner and H. Kunz, *Synlett*, 2000, 400.
106. C.M. Moraes, M.P. Bemquerer and M.T.M. Miranda, *J. Pept. Res*., 2000, **55**, 289.
107. P. Strazzolini, M. Scuccato and A.G. Giumanini, *Tetrahedron*, 2000, **56**, 3625.
108. R.S. Givens, J.F.W. Weber, P.G. Conrad, G. Orosz, S.L. Donahue and S.A. Thayer, *J. Amer. Chem. Soc*., 2000, **122**, 2687.
109. V. Alezra, M. Bonin, A. Chiaroni, L. Micouin, C. Riche and H.-P. Husson, *Tetrahedron Lett*., 2000, **41**, 1737.
110. S. Kurihara, T. Tsumuraya, K. Suzuki, M. Kuroda, L. Liu, Y. Takaoka and I. Fujii, *Chem.-Eur. J*., 2000, **6**, 1656.
111. B.-E. Choi and J.-H. Jeong, *Arch. Pharmacol. Res*., 2000, **23**, 564.
112. Y. Nishiyama, S. Shikama, K.-i. Morita and K. Kurita, *J. Chem. Soc*., *Perkin Trans. 1*, 2000, 1949.
113. Y. Nishiyama, S. Ishizuka, S. Shikama and K. Kurita, *Pept. Sci*., 1999, 91.
114. H. Hojo and Y. Nakahara, *Pept. Sci*., 1999, 97.
115. A.D. Morley, *Tetrahedron Lett*., 2000, **41**, 7401.
116. B.-H. Hu and P.B. Messersmith, *Tetrahedron Lett*., 2000, **41**, 5795.
117. H. Nishio, Y. Nishiuchi, T. Inui, K. Yoshizawa-Kumagaye and T. Kimura, *Tetrahedron Lett*., 2000, **41**, 6839.
118. H. Wang, Z. Miao, L. Lai and X. Xu, *Synth. Commun*., 2000, **30**, 727.
119. A. Karlström, K. Rosenthal and A. Undén, *J. Pept. Res*., 2000, **55**, 36.
120. B. Denis and E. Trefilieff, *J. Pept. Sci*., 2000, **6**, 372.
121. L. Bourel, O. Carion, H. Gras-Masse and O. Melnyk, *J. Pept. Sci*., 2000, **6**, 264.
122. E.V. Kudryavtseva, M.V. Sidorova, M.V. Ovchinnikov and Zh.D. Bespalova, *J. Pept. Sci*., 2000, **6**, 208.
123. A. Schulz, K. Adermann, M. Eulitz, S.M. Feller and C. Kardinal, *Tetrahedron*, 2000, **56**, 3889.
124. T. Shi and D.L. Rabenstein, *J. Amer. Chem. Soc*., 2000, **122**, 6809.
125. R. Soll and A.G. Beck-Sickinger, *J. Pept. Sci*., 2000, **6**, 387.
126. B. Hargittai, I. Annis and G. Barany, *Lett. Pept. Sci*., 2000, **7**, 47.
127. A. Cuthbertson and B. Indrevoll, *Tetrahedron Lett*., 2000, **41**, 3661.
128. Y. Fujiwara, *Yakugaku Zasshi*, 2000, **120**, 197.
129. M. Iwai, H. Yokoyama, H. Yamada, M. Niwa and M. Kobayashi, *Chem. Pharm. Bull*., 2000, **48**, 1304.
130. V.V.S. Babu, K. Ananda and G.-R. Vasanthakumar, *J. Chem. Soc*., *Perkin Trans. 1*, 2000, 4328.
131. I. Grayson, *Spec. Chem*., 2000, **20**, 86.
132. V.V.S. Babu and K. Ananda, *Lett. Pept. Sci*., 2000, **7**, 41.
133. V.V.S. Babu, H.N. Gopi and K. Ananda, *Indian J. Chem*., 2000, **39B**, 291.
134. H.N. Gopi, K. Ananda and V.V.S. Babu, *Protein Pept. Lett*., 2000, **7**, 33.
135. H.N. Gopi and V.V.S.Babu, *Lett. Pept. Sci*., 2000, **7**, 165.
136. A. El-Faham, *Lett. Pept. Sci*., 2000, **7**, 113.
137. L.A. Carpino, H. Imazumi, B.M. Foxman, M.J. Vela, P. Henklein, A. El-Faham, J. Klose and M. Bienert, *Org. Lett*., 2000, **2**, 2253.
138. P. Li and J.-C. Xu, *Chin. J. Chem*., 2000, **18**, 85.
139. P. Li and J.-C. Xu, *Chin. Chem. Lett*., 2000, **11**, 377.
140. P. Li and J.-C. Xu, *Chin. J. Chem*., 2000, **18**, 456.
141. P. Li and J.-C. Xu, *Tetrahedron Lett*., 2000, **41**, 721.
142. P. Li and J.-C. Xu, *Tetrahedron*, 2000, **56**, 4437.
143. P. Li and J.-C. Xu, *Tetrahedron*, 2000, **56**, 8119.

144. P. Li and J.-C. Xu, *Chem. Lett*., 2000, 204.
145. P. Li and J.-C. Xu, *J. Pept. Res*., 2000, **55**, 110.
146. R. Chinchilla, D.J. Dodsworth, C. Nájera and J.M. Soriano, *Tetrahedron Lett*., 2000, **41**, 2463.
147. A. Falchi, G. Giacomelli, A. Porcheddu and M. Taddei, *Synlett*, 2000, 275.
148. Y. Nishiyama and K. Kurita, *Pept. Sci*., 1999, 107.
149. Y. Nishiyama, S. Ishizuka, T. Mori and K. Kurita, *Chem. Pharm. Bull*., 2000, **48**, 442.
150. C. Kashima, S. Tsuruoka and S. Mizuhara, *Heterocycles*, 2000, **52**, 413.
151. W.-R. Li and H.-H. Chou, *Synthesis*, 2000, 84.
152. T. Yasuhara, Y. Nagaoka and K. Tomioka, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2901.
153. Y.-C. Tang, X.-M. Gao, G.-L. Tian and Y.-H. Ye, *Chem. Lett*., 2000, 826.
154. H.-B. Xie, G.-L. Tian and Y.-H. Ye, *Synth. Commun*., 2000, **30**, 4233.
155. Y.-A. Kim and S.-Y. Han, *Bull. Korean Chem. Soc*., 2000, **21**, 943.
156. M. Kurosu, *Tetrahedron Lett*., 2000, **41**, 591.
157. I. Shiina, Y. Suenaga, M. Nakano and T. Mukaiyama, *Bull. Chem. Soc. Jpn*., 2000, **73**, 2811.
158. E. Vedejs and C. Kongkittingam, *J. Org. Chem*., 2000, **65**, 2309.
159. Z. Bozso, G. Toth, R.F. Murphy and S. Lovas, *Lett. Pept. Sci*., 2000, **7**, 157.
160. G.S. Beligere and P.E. Dawson, *Biopolymers*, 2000, **51**, 363.
161. T. Kawakami, K. Hasegawa, K. Teruya, K. Akaji, M. Horiuchi, F. Inagaki, Y. Kurihara, S. Uesugi and S. Aimoto, *Pept. Sci*., 1999, 111.
162. B. Ayers, U.K. Blaschke, J.A. Camarero, G.J. Cotton, M. Holford and T.W. Muir, *Biopolymers*, 2000, **51**, 343.
163. A.K. Mapp and P.B. Dervan, *Tetrahedron Lett*., 2000, **41**, 9451.
164. A. Brik, E. Keinan and P.E. Dawson, *J. Org. Chem*., 2000, **65**, 3829.
165. J. Offer and P.E. Dawson, *Org. Lett*., 2000, **2**, 23.
166. B.L. Nilsson, L.L. Keissling and R.T. Raines, *Org. Lett*., 2000, **2**, 1939.
167. P. Ajikumar and K.S. Devaky, *Proc. -Indian Acad. Sci., Chem. Sci*., 2000, **112**, 465.
168. M. Roice, K.S. Kumar and V.N.R. Pillai, *Protein Pept. Lett*., 2000, **7**, 151.
169. C. Arunan and V.N.R. Pillai, *Tetrahedron*, 2000, **56**, 3005.
170. M. Roice, K.S. Kumar and V.N.R. Pillai, *Tetrahedron*, 2000, **56**, 3725.
171. J.K. Cho, B.-D. Park and Y.-S. Lee, *Tetrahedron Lett*., 2000, **41**, 7481.
172. T. Groth, M. Grotli, W.D. Lubell, L.P. Miranda and M. Meldal, *J. Chem. Soc., Perkin Trans. 1*, 2000, 4258.
173. W.A. Neugebauer, P. D'Orleans-Juste and G. Bkaily, *Adv. Chitin Sci*., 2000, **4**, 411.
174. K. Licha, S. Bhargava, C. Rheinlander, A. Becker, J. Schneider-Mergener and R. Volkmer-Engert, *Tetrahedron Lett*., 2000, **41**, 1711.
175. I.A. Rivero and R. Aceves, *Rec. Soc. Quim. Mex*., 2000, **44**, 97.
176. C.T. Bui, F. Ercole, Y. Pham, R. Campbell, F.A. Rasoul, N.J. Maeji and N.J. Ede, *J. Pept. Sci*., 2000, **6**, 534.
177. C.T. Bui, A.M. Bray, T. Nguyen, F. Ercole, F. Rasoul, W. Sampson and N.J. Maeji, *J. Pept. Sci*., 2000, **6**, 49.
178. C.T. Bui, A.M. Bray, T. Nguyen, F. Ercole and N.J. Maeji, *J. Pept. Sci*., 2000, **6**, 243.
179. A. Svensson, T. Fex and J. Kihlberg, *J. Comb. Chem*., 2000, **2**, 736.
180. G.E. Atkinson, P.M. Fischer and W.C. Chan, *J. Org. Chem*., 2000, **65**, 5048.
181. K.J. Jensen, J. Alsina, M.F. Songster, J. Vágner, F. Albericio and G. Barany, *J. Amer. Chem. Soc*., 1998, **120**, 5441.
182. J. Alsina, T.S. Yokum, F. Albericio and G. Barany, *Tetrahedron Lett*., 2000, **41**,

7277.
183. J.A. Camarero, A. Adeva and T.W. Muir, *Lett. Pept. Sci.*, 2000, **7**, 17.
184. A.S. Goldstein and M.H. Gelb, *Tetrahedron Lett.*, 2000, **41**, 2797.
185. T. Okayama, A. Burritt and V.J. Hruby, *Org. Lett.*, 2000, **2**, 1787.
186. A. Ishii, H. Hojo, A. Kobayashi, K. Nakamura, Y. Nakahara, Y. Ito and Y. Nakahara, *Tetrahedron*, 2000, **56**, 6235.
187. Y. Nakahara, S. Ando, M. Itakura, N. Kumabe, H. Hojo, Y. Ito and Y. Nakahara, *Tetrahedron Lett.*, 2000, **41**, 6489.
188. K.C. Nicolaou, N. Winssinger, R. Hughes, C. Smethurst and S.Y. Cho, *Angew. Chem., Int. Ed.*, 2000, **39**, 1084.
189. L.P. Miranda, W.D.F. Meutermans, M.L. Smythe and P.F. Alewood, *J. Org. Chem.*, 2000, **65**, 5460.
190. J. Howe, M. Quibell and T. Johnson, *Tetrahedron Lett.*, 2000, **41**, 3997.
191. D. Swinnen and D. Hilvert, *Org. Lett.*, 2000, **2**, 2439.
192. A.B. Clippingdale, C.J. Barrow and J.D. Wade, *J. Pept. Sci.*, 2000, **6**, 225.
193. C.W. Tornoe, P. Davis, F. Porreca and M. Meldal, *J. Pept. Sci.*, 2000, **6**, 594.
194. F. Guillaumie, J.C. Kappel, N.M. Kelly, G. Barany and K.J. Jensen, *Tetrahedron Lett.*, 2000, **41**, 6131.
195. T. Johnson, M. Liley, T.J. Cheeseright and F. Begum, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2811.
196. F. Rabanal, J.J. Pastor, E. Nicolás, F. Albericio and E. Giralt, *Tetrahedron Lett.*, 2000, **41**, 8093.
197. J.D. Wade, M.N. Mathieu, M. Macris and G.W. Tregear, *Lett. Pept. Sci.*, 2000, **7**, 107.
198. Y. Nishiuchi, H. Nishio, T. Inui, K. Yoshizawa-Kumagaye and T. Kimura, *Pept. Sci.*, 1999, 105.
199. M. Rinnova, M. Novakova, V. Kasicka and J. Jirasek, *J. Pept. Sci.*, 2000, **6**, 355.
200. M. Liley and T. Johnson, *Tetrahedron Lett.*, 2000, **41**, 3983.
201. Yu.A. Rubina, Zh.D. Bespalova and V.N. Bushuev, *Russ. J. Bioorg. Chem.* , 2000, **26**, 235.
202. M. del Fresno, A. El-Faham, L.A. Carpino, M. Royo and F. Albericio, *Org. Lett.*, 2000, **2**, 3539.
203. A.K. Ghosh and E. Fan, *Tetrahedron Lett.*, 2000, **41**, 165.
204. D. Shan, A. Zheng, C.E. Ballard, W. Wang, R.T. Borchardt and B. Wang, *Chem. Pharm. Bull.*, 2000, **48**, 238.
205. M.A. Shogren-Knaak, K.A. McDonnell and B. Imperiali, *Tetrahedron Lett.*, 2000, **41**, 827.
206. Y. Nishiuchi, H. Nishio, T. Inui, J. Bodi and T. Kimura, *J. Pept. Sci.*, 2000, **6**, 84.
207. N. Thieriet, F. Guibé and F. Albericio, *Org. Lett.*, 2000, **2**, 1815.
208. W.-R. Li, S.T. Lin and J.H. Yang, *Synlett*, 2000, 1608.
209. W.-R. Li, Y.-C. Yo and Y.-S. Lin, *Tetrahedron*, 2000, **56**, 8867.
210. Y. Lee and R.B. Silverman, *Org. Lett.*, 2000, **2**, 303.
211. N. Sugimoto, D. Miyoshi and J. Zou, *Chem. Commun.*, 2000, 2295.
212. H. De Muynck, A. Madder, N. Farcy, P.J. De Clercq, M.N. Pérez-Payán, L.M. Öhberg and A.P. Davis, *Angew. Chem., Int. Ed.*, 2000, **39**, 145.
213. G. Haberhauer, L. Somogyi and J. Rebek, *Tetrahedron Lett.*, 2000, **41**, 5013.
214. A. Graven, M. Grotli and M. Meldal, *J. Chem. Soc., Perkin Trans. 1*, 2000, 955.
215. R. Afrin, T. Haruyama, Y. Yanagida, E. Kobetake and M. Aizawa, *J. Mol. Catal. B: Enzym.*, 2000, **9**, 259.
216. M. Miyanaga, K. Imamura, T. Sakiyama, K. Nakanishi, *J. Biosci. Bioeng.*, 2000, **90**,

112.
217. L.M. van Langen, F. van Rantwijk, V.K. Švedas and R.A. Sheldon, *Tetrahedron: Asymmetry*, 2000, **11**, 1077.
218. A. Basso, L. De Martin, C. Ebert, L. Gardossi and P. Linda, *Chem. Commun*., 2000, 467.
219. R.V. Ulijn, M. Erbeldinger and P.J. Halling, *Biotechnol. Bioeng*., 2000, **69**, 633.
220. M.L.M. Serralheiro and J.M.S. Cabral, *Biocatal. Biotransform*., 1999, **17**, 3.
221. A.V. Vakurov, A.K. Gladilin, J. Partridge, V.A. Izumrudov, V.V. Mozhaev, A.V. Levashov and P.J. Halling, *Biocatal. Biotransform*., 1999, **17**, 283.
222. S.-y. Okazaki, M. Goto and S. Furusaki, *Enzyme Microb. Technol*., 2000, **26**, 159.
223. G.-W. Xing, X.-W. Li, G.-L. Tian and Y.-H. Ye, *Tetrahedron*, 2000, **56**, 3517.
224. S. Kumaran, D. Datta and R.P. Roy, *Protein Sci*., 2000, **9**, 734.
225. J.-E. So, J.-S. Shin and B.-G. Kim, *Enzyme Microb. Technol*., 2000, **26**, 108.
226. L.M. Simon, M. Kotorman, K. Maraczi and K. Laszlo, *J. Mol. Catal. B: Enzym*., 2000, **10**, 565.
227. A. Trusek-Holowinia and A. Noworytz, *Biotechnologia*, 2000, 156.
228. M. Erbeldinger, A.J. Mesiano and A.J. Russell, *Biotechnol. Prog*., 2000, **16**, 1131.
229. M. Miyanaga, M. Ohmori, K. Imamura, T. Sakiyama and K. Nakanishi, *J. Biosci. Bioeng*., 2000, **90**, 43.
230. S. Rival, J. Saulnier and J. Wallach, *Biocatal. Biotransform*., 2000, **17**, 417.
231. J.U. Klein, A. Prykhodzka and V. Cerovsky, *J. Pept. Sci*., 2000, **6**, 541.
232. H. Ogino, M. Yamada, F. Watanabe, H. Ichinose, M. Yasuda and H. Ishikawa, *J. Biosci. Bioeng*., 1999, **88**, 513.
233. H. Ogino, Y. Gemba, M. Yamada, M. Shizuka, M. Yasuda and H. Ishikawa, *Biochem. Eng. J*., 2000, **5**, 219.
234. Y. Isono and M. Nakajima, *Biochem. Eng. J*. , 2000, **4**, 143.
235. Y. Isono and N. Nakajima, *Process Biochem*, 2000, **36**, 275.
236. K. Mishima and K. Matsuyama, *Chorinkai Saishin Gijitsu*, 2000, **4**, 80.
237. J. Kim, D.S. Clark and J.S. Dordick, *Biotechnol. Bioeng*., 2000, **67**, 112.
238. S.V. Kolobanova, I.Yu. Filippova, E.N. Lysogorskaya, I.V. Getun, A.V. Bacheva, E.S. Oksenoit and V.M. Stepanov, *Russ. J. Bioorg. Chem*., 2000, **26**, 369.
239. P. Bjorup, P. Adlercreutz and P. Clapes, *Biocatal. Biotransform*., 1999, **17**, 319.
240. N. Kato, A. Oishi and F. Takahashi, *Mater. Sci. Eng*., 2000, **C13,** 109.
241. M. Haensler, H.-D. Wissmann and N. Wihofsky, *J. Pept. Sci*., 2000, **6**, 366.
242. N. Wehofsky, M. Haensler, S.W. Kirbach, J.-D. Wissmann and F. Bordusa, *Tetrahedron: Asymmetry*, 2000, **11**, 2421.
243. N. Wehofsky, S.W. Kirbach, M. Haensler, J.-D. Wissmann and F. Bordusa, *Org. Lett*., 2000, **2**, 2027.
244. M. Haensler and K. Arnold, *Biol. Chem*., 2000, **381**, 79.
245. V. Čeřovský and F. Bordusa, *J. Pept. Res*., 2000, **55**, 325.
246. T. Miyazawa, S. Masaki, R. Yanagihara and T. Yamada, *Pept. Sci*., 1999, 133.
247. N. Wehofsky, J.-D. Wissmann, M. Alisch and F. Bordusa, *Biochim. Biophys. Acta*, 2000, **1479**, 114.
248. K. Braun, Yu.V. Mitin, K. Salchert, T. Schmidt and P. Kuhl, *Biocatal. Biotransform*., 2000, **18**, 427.
249. R. Günther and F. Bordusa, *Chem.-Eur. J*., 2000, **6**, 463.
250. R. Günther, A. Stein and F. Bordusa, *J. Org. Chem*., 2000, **65**, 1672.
251. H. Sekizaki, M. Murakami, K. Itoh, E. Toyota and K. Tanizawa, *J. Mol. Catal. B: Enzym*., 2000, **11**, 23.
252. R. Günther, S. Thust, H.-J. Hofmann and F. Bordusa, *Eur. J. Biochem*., 2000, **267**,

3496.
253. H. Takagi, K. Hirai, Y. Maeda, H. Matsuzawa and S. Nakamori, *J. Biochem.*, 2000, **127**, 617.
254. R.J. Elliott, A.J. Bennet, C.A. Braun, A.M. MacLeod and T.J. Borgford, *Chem. Biol.*, 2000, **7**, 163.
255. T. Kappes-Roth and H. Waldmann, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2579.
256. A.G. Gum, T. Kappes-Roth and H. Waldmann, *Chem. -Eur. J.*, 2000, **6**, 3714.
257. J.-S. Shin, S. Luque and A.M. Klibanov, *Biotechnol. Bioeng.*, 2000, **69**, 577.
258. H. Kanzaki, D. Imura, T. Nitoda and K. Kawazu, *J. Biosci. Bioeng.*, 2000, **90**, 86.
259. V. Čeřovský, J. Kockskamper, H.G. Glitsch and F. Bordusa, *ChemBioChem.*, 2000, **1**, 126.
260. G. Espuña, G. Arsequell, G. Valencia, G. Barluenga, M. Pérez and J.M. González, *Chem. Commun.*, 2000, 1307.
261. V. Tolmachev, A. Orlova and H. Lundqvist, *J. Radioanal. Nucl. Chem.*, 2000, **246**, 207.
262. C.F. Foulon, C.J. Reist, D.D. Bigner and M.R. Zalutsky, *Cancer Res.*, 2000, **60**, 4453.
263. M. Ricci, L. Madariaga and T. Skrydstrup, *Angew. Chem., Int. Ed.*, 2000, **39**, 242.
264. M. Ricci, P. Blakskjaer and T. Skrydstrup, *J. Amer. Chem. Soc.*, 2000, **122**, 12413.
265. S.-I. Murahashi, A. Mitani and K. Kitao, *Tetrahedron Lett.*, 2000, **41**, 10245.
266. R.T. Buck, P.A. Clarke, D.M. Coe, M.J. Drysdale, L. Ferris, D. Haigh, C.J. Moody, N.D. Pearson and E. Swann, *Chem.-Eur. J.*, 2000, **6**, 2160.
267. X. Li, L. Zhang, S.E. Hall and J.P. Tam, *Tetrahedron Lett.*, 2000, **41**, 4069.
268. K. Nokihara, T. Yasuhara, A. Blahunka, T. Kasama and V. Wray, *Pept. Sci.*, 1999, 101.
269. N. Valls, M. Lopez-Canet, M. Vallribera and J. Bonjoch, *J. Amer. Chem. Soc.*, 2000, **122**, 11248.
270. F. Yokokawa, K. Izumi, J. Omata and T. Shioiri, *Tetrahedron*, 2000, **56**, 3027.
271. S. Ohnishi, A. Koide and S. Koide, *J. Mol. Biol.*, 2000, **301**, 477.
272. T. Inui, H. Nishio, Y. Nishiuchi and T. Kimura, *Pept. Sci.*, 1999, 5.
273. O.M.A. El-Agnaf, H. Goodwin, J.M. Sheridan, E.R. Frears and B.M. Austen, *Protein Pept. Lett.*, 2000, **7**, 1.
274. J.M. Matsoukas, L. Polevaya, J. Ancans, T. Mavromoustakos, A. Kolocouris, P. Roumelioti, D.V. Vlahakos, R. Yamdagni, Q. Wu and G.J. Moore, *Bioorg. Med. Chem.*, 2000, **8**, 1.
275. Y.-I. Takahashi, O. Fukuo, R. Akasaka, N. Izuhara, K. Yamada, H. Yamamura, S. Araki, M. Kawai, K. Saito and F. Mukai, *Pept. Sci.*, 1999, 391.
276. H. Sasaki and M. Sisido, *Pept. Sci.*, 1999, 355.
277. H. Kodama, M. Shibue, H. Yamaguchi, T. Hara, M. Jelokhani- Niaraki and M. Kondo, *Pept. Sci.*, 1999, 445.
278. E. Freund, F. Vitali, A. Linden and J.A. Robinson, *Helv. Chim. Acta*, 2000, **83**, 2572.
279. C.J. Leitheiser, M.J. Rishel, X. Wu and S.M. Hecht, *Org. Lett.*, 2000, **2**, 3397.
280. S.K. Sharma, M. Tandon and J.W. Lown, *Eur. J. Org. Chem.*, 2000, 2095.
281. T. Niidome, M. Tsuiki, Y. Tokunaga, T. Hatakeyama and H. Aoyagi, *Bull. Chem. Soc. Jpn.*, 2000, **73**, 1397.
282. J.P. Tam, C. Wu and J.-L. Yang, *Eur. J. Biochem.*, 2000, **267**, 3289.
283. P.A. Raj, K.J. Antonyraj and T. Karunakaran, *Biochem. J.*, 2000, **347**, 633.
284. M. Cudic, P. Bulet and L. Otvos, *Protein Pept. Lett.*, 2000, **7**, 143.
285. D.L. Boger, B.E. Fink and M.P. Hedrick, *J. Amer. Chem. Soc.*, 2000, **122**, 6382.
286. S. Bhattacharya and M. Thomas, *Tetrahedron Lett.*, 2000, **41**, 5571.

287. M. Cudic, J.D. Wade and L. Otvos, *Tetrahedron Lett*., 2000, **41**, 4527.
288. S. Burrage, T. Raynham, G. Williams, J.W. Essex, C. Allen, M. Cardno, V. Swali and M. Bradley, *Chem.-Eur. J*., 2000, **6**, 1455.
289. S. Armaroli, G. Cardillo, L. Gentilucci, M. Gianotti and A. Tolomelli, *Org. Lett*., 2000, **2**, 1105.
290. M.C. Bagley, K.E. Bashford, C.L. Hesketh and C.J. Moody, *J. Amer. Chem. Soc*., 2000, **122**, 3301.
291. S. Rana, J. Dubuc, M. Bradley and P. White, *Tetrahedron Lett*., 2000, **41**, 5135.
292. P.A. Raj, T. Karunakaran and D.K. Sukumaran, *Biopolymers*, 2000, **53**, 281.
293. C. Peggion, C. Piazza, F. Formaggio, M. Crisma, C. Toniolo, B. Kaptein, Q.B. Broxterman and J. Kamphuis, *Lett. Pept. Sci*., 2000, **7**, 9.
294. K. Konishi, K. Ikeda, K. Achiwa, H. Hoshino and K. Tanaka, *Chem. Pharm. Bull*., 2000, **48**, 308.
295. H. Tamamura, A. Omagari, S. Oishi, T. Kanamoto, N. Yamamoto, S.C. Peiper, H. Nakashima, A. Otaka and N. Fujii, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2633.
296. F. Hubler, T. Rückle, L. Patiny, T. Muamba, J.-F. Guichou, M. Mutter and R. Wenger, *Tetrahedron Lett*., 2000, **41**, 7193.
297. Y. Mukai, T. Niidome, T. Hatakeyama and H. Aoyagi, *Pept. Sci*., 1999, 451.
298. S. Sriyastava, G.C. Melkani, S. Singh and V.D. Gupta, *Indian J. Biochem. Biophys*., 2000, **37**, 318.
299. T.M. Hackeng, J.A. Fernandez, P.E. Dawson, S.B.H. Kent and J.H. Griffin, *Proc. Natl. Acad. Sci., U.S.A*., 2000, **97**, 14074.
300. M. Cristau, C. Devin, C. Oiry, O. Chaloin, M. Amblard, N. Bernad, A. Heitz, J.-A. Fehrentz and J. Martinez, *J. Med. Chem*., 2000, **43**, 2356.
301. P. Bedos, M. Amblard, G. Subra, P. Dodey, J.-M. Luccarini, J.-L. Paquet, D. Pruneau, A. Aumelas and J. Martinez, *J. Med. Chem*., 2000, **43**, 2387.
302. L. Quartara, R. Ricci, S. Meini, R. Patacchini, A. Giolitti, S. Amadesi, C. Rizzi, A. Rizzi, K. Varani, P.A. Borea, C.A. Maggi and D. Regoli, *Eur. J. Med. Chem*., 2000, **35**, 1001.
303. M. Heller, K.M. Loomes and C.J.S. Cooper, *Anal. Biochem*., 2000, **285**, 100.
304. Y. Zhang, W. Wu, M. Wang and T. Wu, *Zhongguo Yaowu Huaxue Zazhi*, 2000, **10**, 124.
305. A.J. DeLucca, J.M. Bland, C.B. Vigo, T.J. Jacks, J. Peter and T.J. Walsh, *Med. Mycol*., 2000, **38**, 301.
306. S.Y. Shin, J.H. Kang, S.Y. Jang, Y. Kim, K.L. Kim and K.S. Hahm, *Biochim. Biophys. Acta*, 2000, **1463**, 209.
307. D. Oh, S.Y. Shin, S. Lee, J.H. Kang, S.D. Kim, P.D. Ryu, K.- S. Hahm and Y. Kim, *Biochemistry*, 2000, **39**, 11855.
308. M.C. Pirrung, S.J. Drabik, J. Ahamed and H. Ali, *Bioconjugate Chem*., 2000, **11**, 679.
309. J.S. Davies, C. Enjalbal, C. Nguyen, L. Al-Jamri and C. Naumer, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2907.
310. C.W. Tornoe, H. Sengelov and M. Meldal, *J. Pept. Sci*., 2000, **6**, 314.
311. G.P. Zecchini, M.P. Paradisi, I. Torrini, M. Nalli, G. Lucente and S. Spisani, *Farmaco*, 2000, **55**, 308.
312. I. Torrini, M.P. Paradisi, G.P. Zechini, G. Lucente, G. Mastropietro and S. Spisani, *J. Pept. Res*., 2000, **55**, 102.
313. L.P. Miranda, T. Tao, A. Jones, I. Chernushevich, K.G. Standing, C.L. Geczy and P,F. Alewood, *FEBS Lett*., 2000, **488**, 85.
314. M. Martín-Martínez, N. De la Figuera, M. Latorre, R. Herranz, M.-T. García-López, E. Cenarruzabietia, J. Del Rio and R. Gonzalez-Muniz, *J. Med. Chem*., 2000,

43, 3770.
315. C.M.R. Low, J.W. Black, H.B. Broughton, I.M. Buck, J.M.R. Davies, D.J. Gunstone, R.A.D. Hull, S.B. Kalindjian, I.M. McDonald, M.J. Pether, N.P. Shankley and K.I.M. Steele, *J. Med. Chem.*, 2000, **43**, 3505.
316. K. Kitagawa, H. Adachi, H. Fujiwara, S. Futaki and T. Yagami, *Pept. Sci.*, 1999, 113.
317. B. Bellier, M.-E. Million, S. DaNascimento, H. Meudal, S. Kellou, B. Maigret and C. Garbay, *J. Med. Chem.*, 2000, **43**, 3614.
318. J.A. Malone, R.D. Reidelberger and H. Hulce, *J. Labelled Compd. Radiopharm.*, 2000, **43**, 77.
319. D.E. Mechling and H.P. Bächinger, *J. Biol. Chem.*, 2000, **275**, 14532.
320. Y.-S. Yang, G. Mitta, A. Chavanieu, B. Calas, J.F. Sanchez, P. Roch and A. Aumelas, *Biochemistry*, 2000, **39**, 14436.
321. N.F. Dawson, D.J. Craik, A.M. Mcmanus, S.G. Dashper, E.C. Reynolds, G.W. Tregear, L. Otvos and J.D. Wade, *J. Pept. Sci.*, 2000, **6**, 19.
322. B. Liang, M.D. Vera and M.M. Joullié, *J. Org. Chem.*, 2000, **65**, 4762.
323. P. Portonovo, X. Ding, M.S. Leonard and M.M. Joullié, *Tetrahedron*, 2000, **56**, 3687.
324. M. Martino, A. Coviello and A.M. Tamburro, *Int. J. Biol. Macromol.*, 2000, **27**, 59.
325. R.A. McMillan and V.P. Conticello, *Macromolecules*, 2000, **33**, 4809.
326. M. Falorni, G. Giacomelli, A. Porcheddu and G. Dettori, *Eur. J. Org. Chem.*, 2000, 3217.
327. M. Karasawa, K. Kobayashi, H. Oku, K. Sato, S. Kano, M. Suzuki and R. Katakai, *Pept. Sci.*, 1999, 295.
328. J. Satyanarayana, H. Situ, S. Narasimhamurthy, N. Bhayani, L.A. Bobek and M.J. Levine, *J. Pept. Res.*, 2000, **56**, 275.
329. J. Jamison, S. Levy, X. Sun, D. Zeckner, W. Current, M. Zweifel, M. Rodriguez, W. Turner and S.-H. Chen, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2101.
330. S.-H. Chen, X. Sun, R. Boyer, J. Paschal, D. Zeckner, W. Current, M. Zweifel and M. Rodriguez, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2107.
331. L. Balaspiri, Z. Tegyei, M. Dux, G. Jancso, R. Jozsa, V. Csernus and B. Mess, *Lett. Pept. Sci.*, 2000, **7**, 23.
332. T. Itoh, M. Miwa, M. Suenaga, T. Ohtaki, C. Kitada, O. Nishimura and M. Fujino, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1333.
333. T. Itoh, M. Suenaga, M. Miwa, R. Fujii, T. Ohtaki, C. Kitada, O. Nishimura and M. Fujino, *Pept. Sci.*, 1999, 167.
334. D. Trivedi, Y. Lin, J.-M. Ahn, M. Siegel, N.N. Mollova, K.H. Schram and V.J. Hruby, *J. Med. Chem.*, 2000, **43**, 1714.
335. L.B. Knudsen, P.F. Nielsen, P.O. Huusfeldt, N.L. Johansen, K. Madsen, F.Z. Pedersen, H. Thøgersen, M. Wilken and H. Agersø, *J. Med. Chem.*, 2000, **43**, 1664.
336. I. Kato, L. Jun, K. Kitamura, H. Tada, N. Yanaihara, Y. Hirotani, K. Yamamoto, N. Kurokawa and C. Yanaihara, *Pept. Sci.*, 1999, 159.
337. J. Hlavacek, B. Bennettova, R. Tykva, J. Velek, V. Kasicka and T. Barth, *Lett. Pept. Sci.*, 2000, **7**, 85.
338. M. Cavero, A. Hobbs, D. Madge, W.B. Motherwell, D. Selwood and P. Potier, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 641.
339. D. Yahalom, S. Rahimipour, Y. Koch, N. Ben-Aroya and M. Fridkin, *J. Med. Chem.*, 2000, **43**, 2824, 2831.
340. J.E. Rivier, R.S. Struthers, J. Porter, S.L. Lahrichi, G. Jiang, L.A. Cervini, M. Ibea, D.A. Kirby, S.C. Koerber and C.L. Rivier, *J. Med. Chem.*, 2000, **43**, 784.

341. J.E. Rivier, G. Jiang, R.S. Struthers, S.C. Koerber, J. Porter, L.A. Cervini, D.A. Kirby, A.G. Craig and C.L. Rivier, *J. Med. Chem.*, 2000, **43**, 807.
342. J.E. Rivier, J. Porter, L.A. Cervini, S.L. Lahrichi, D.A. Kirby, R.S. Struthers, S.C. Koerber and C.L. Rivier, *J. Med. Chem.*, 2000, **43**, 797.
343. R. Tacke, M. Merget, R. Bertermann, M. Bernd, T. Beckers and T. Reissmann, *Organometallics*, 2000, **19**, 3486.
344. R. Fang, C.-D. Zhang, L.-P. Wang, H. Zhou and W. Li, *Chem. Res. Chin. Univ.*, 2000, **16**, 122.
345. B. Peschke, M. Ankersen, T.K. Hansen, B.S. Hansen, J. Lau, K.K. Nielsen and K. Raun, *Eur. J. Med. Chem.*, 2000, **35**, 599.
346. Z. Rekasi, J.L. Varga, A.V. Schally, G. Halmos, K. Groot and T. Czomploy, *Proc. Natl. Acad. Sci.*, 2000, **97**, 1218.
347. N. Kashihara, S. To-E, K. Nakamura, K. Umezawa, S. Yamamura and S. Nishiyama, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 101.
348. A. Velentza, S. Spiliou, C.P. Poulos and G.J. Goldsworthy, *Peptides*, 2000, **21**, 631.
349. J.B. Kristensen, L.K. Muller and U.D. Larsen, *J. Labelled Compd. Radiopharm.*, 2000, **43**, 671.
350. X. Dai, Z. Su and J.O. Liu, *Tetrahedron Lett.*, 2000, **41**, 6295.
351. S.C. Archibald, J.C. Head, N. Gozzard, D.W. Howat, T.A.H. Parton, J.R. Porter, M.K. Robinson, A. Shock, G.J. Warrellow and W.M. Abraham, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 997.
352. S.C. Archibald, J.C. Head, J.M. Linsley, J.R. Porter, M.K. Robinson, A. Shock and G.J. Warrellow, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 993.
353. A.S. Dutta, M. Crowther, J.J. Gormley, L. Hassall, C.F. Hayward, P.R. Gellert, R.S. Kittlety, P.J. Alcock, A. Jamieson, J.M. Moores, A. Rees, L.J. Wood, C.F. Reilly and D. Haworth, *J. Pept. Sci.*, 2000, **6**, 321.
354. B.P. England, P. Balasubramanian, I. Uings, S. Bethell, M.- J. Chen, P.J. Schatz, Q. Yin, Y.-F. Chen, E.A. Whitehorn, A. Tsavaler, C.L. Martens, R.W. Barrett and M. McKinnon, *Proc. Natl. Acad. Sci., U.S.A.*, 2000, **97**, 6862.
355. B. Anichini, R. Ricci, G. Fabbri, G. Balacco, S. Mauro, A. Triolo, M. Altamura, C.A. Maggi and L. Quartara, *J. Pept. Sci.*, 2000, **6**, 612.
356. K. Hojo, M. Maeda, Y. Mu, H. Kamada, Y. Tsutsumi, Y. Nishiyama, T. Yoshikawa, K. Kurita, L.H. Block, T. Mayumi and K. Kawasaki, *J. Pharm. Pharmacol.*, 2000, **52**, 67.
357. Y. Nishiyama, T. Yoshikawa, N. Ohara, K. Kurita, K. Hojo, H. Kamada, Y. Tsutsumi, T. Mayumi and K. Kawasaki, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1161.
358. S. Zoffmann, G. Turcatti, J.-L. Galzi, M. Dahl and M. Chollet, *J. Med. Chem.*, 2001, **44**, 215.
359. C. Boss, P.H. Rasmussen, A.R. Wartini and S.R. Waldvogel, *Tetrahedron Lett.*, 2000, **41**, 6327.
360. C.M. Taylor and C.A. Weir, *J. Org. Chem.*, 2000, **65**, 1414.
361. P. Wipf and Y. Uto, *J. Org. Chem.*, 2000, **65**, 1037.
362. C.A. Veale, V.C. Alford, D. Aharony, D.L. Banville, R.A. Bialecki, F.J. Brown, J.R. Damewood, C.L. Dantzman, P.D. Edwards, R.T. Jacobs, R.C. Mauger, M.M. Murphy, W.E. Palmer, K.K. Pine, W.L. Rumsey, L.E. Garcia-Davenport, A. Shaw, G.B. Steelman, J.M. Surian and E.P. Vacek, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1949.
363. J.T. Lundquist, E.E. Büllesbach and T.A. Dix, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 453.
364. N. Sakura, K. Kurosawa and T. Hashimoto, *Chem. Pharm. Bull.*, 2000, **48**, 1166.

365. G. Giraudi, C. Giovannoli, C. Tozzi, C. Baggiani and L. Anfossi, *Chem. Commun.*, 2000, 1135.
366. L. Longobardo, D. Melck, R. Siciliano, A. Santini, V. Di Marzo and G. Cammarota, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1185.
367. S.F. Martin, M.P. Dwyer, B. Hartmann and K.S. Knight, *J. Org. Chem.*, 2000, **65**, 1305.
368. M. Anna Rosei, R. Coccia, C. Foppoli, C. Blarzino, C. Cini and M.E. Schinina, *Biochim. Biophys Acta*, 2000, **1478**, 19.
369. Z. Darula, K.E. Kövér, K. Monory, A. Borsodi, E. Makó, A. Rónai, D. Tourwé, A. Péter and G. Tóth, *J. Med. Chem.*, 2000, **43**, 1359.
370. Z.S. Arnold and P.W. Schiller, *J. Pept. Sci.*, 2000, **6**, 280.
371. G. Cardillo, L. Gentilucci, P. Melchiorre and S. Spampinato, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2755.
372. A. Olma and D. Tourwe, *Lett. Pept. Sci.*, 2000, **7**, 93.
373. K. Okada, T. Sujaku, Y. Chuman, R. Nakashima, T. Nose, T. Costa, Y. Yamada, M. Yokoyama, A. Nagahisa and Y. Shimohigashi, *Biochem. Biophys. Res. Commun.*, 2000, **278**, 493.
374. D. Pagé, A. McClory, T. Mischki, R. Schmidt, J. Butterworth, S. St-Onge, M. Labarre, K. Payza and W. Brown, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 167.
375. D.Y. Maeda, F. Berman, T.F. Murray and J.V. Aldrich, *J. Med. Chem.*, 2000, **43**, 5044.
376. V. Kumar, T.F. Murray and J.V. Aldrich, *J. Med. Chem.*, 2000, **43**, 5050.
377. D.Y. Maeda, J.E. Ishmael, T.F. Murray and J.V. Aldrich, *J. Med. Chem.*, 2000, **43**, 3941.
378. L. Leelasvatanakij and J.V. Aldrich, *J. Pept. Res.*, 2000, **56**, 80.
379. P.W. Schiller, T.M.-D. Nguyen, I. Berezowska, S. Dupuis, G. Weltrowska, N.N. Chung and C. Lemieux, *Eur. J. Med. Chem.*, 2000, **35**, 895.
380. G.G. Bonner, P. Davis, D. Stropova, S. Edsall, H.I. Yamamura, F. Porreca and V.J. Hruby, *J. Med. Chem.*, 2000, **43**, 569.
381. Y.-C. Chen, I. Bab, N. Mansur, A. Muhlrad, A. Shteyer, M. Namdar-Attar, H. Gavish, M. Vidson and M. Chorev, *J. Pept. Res.*, 2000, **56**, 147.
382. J.-R. Barbier, S. MacLean, P. Morley, J.F. Whitfield and G.E. Willick, *Biochemistry*, 2000, **39**, 14522.
383. H Xie, Y. Shao, J.M. Becker, F. Naider and R.A. Gibbs, *J. Org. Chem.*, 2000, **65**, 8552.
384. K. Strømgaard, T.J. Brier, K. Andersen, I.R. Mellor, A. Saghyan, D. Tikhonov, P.N.R. Usherwood, P. Krogsgaard- Larsen and J.W. Jaroszewski, *J. Med. Chem.*, 2000, **43**, 4526.
385. L. Bélec, J. Slaninova and W.D. Lubell, *J. Med. Chem.*, 2000, **43**, 1448.
386. G.K. Tóth, Z. Kele and F. Fülöp, *Tetrahedron Lett.*, 2000, **41**, 10095.
387. M. Kempe, *Lett. Pept. Sci.*, 2000, **7**, 27.
388. Y.-M. Zhou and C.-Q. Chen, *Zhongguo Yiyao Gongye Zazhi*, 2000, **31**, 53.
389. M.C. Evans and R.L. Johnson, *Tetrahedron*, 2000, **56**, 9801.
390. I. Wirkus-Romanowska, S. Rodziewicz-Motowidlo, H. Miecznikowska, K. Rolka, M. Janusz, S. Szymaniec, A. Zablocka, W. Fortuna, R. Miedzybrodzki, J. Lisowski and G. Kupryszewski, *Pol. J. Chem.*, 2000, **74**, 1129.
391. I. Wirkus-Romanowska, H. Miecznikowska, M. Janusz, S. Szymaniec, W. Fortuna, R. Miedzybrodzki, A. Zablocka, J. Lisowski and G. Kupryszewski, *Pol. J. Chem.*, 2000, **74**, 979.
392. N.M. Mathieu, J.D. Wade, Y.-Y. Tan, R.J. Summers and G.W. Tregear, *J. Pept.*

Sci., 2000, **6**, 235.
393. A.A. Krys'ko, V.M. Kabanov, P.G. Polishchuk, B.M. Chugunov, V.I. Pavlovskii, S.A. Adronati, A.V. Mazepa, T.A. Kabanova and T.L. Karaseva, *Russ. J. Gen. Chem*., 2000, **70**, 948.
394. A.Dal. Pozzo, M. Fagnoni, R. Bergonzi, L. Vanini, R. de Castiglione, C. Aglio and S. Colli, *J. Pept. Res*., 2000, **55**, 447.
395. Z. Ming, W. Chao, Y. Jian and P. Shiqi, *Prep. Biochem. Biotechnol*., 2000, **30**, 247.
396. K. Akaji and S. Aimoto, *Pept. Sci*., 1999, 1.
397. A. Arendt, J.H. McDowell, W.C. Smith and P.A. Hargrave, *Protein Pept. Lett*., 2000, **7**, 359.
398. H. Li, X. Jiang, S.B. Howell and M. Goodman, *J. Pept. Sci*., 2000, **6**, 26.
399. C. Arunan, R. Nagaraj and V.N.R. Pillai, *Peptides*, 2000, **21**, 773.
400. D. Besser, B. Muller, P. Kleinwachter, G. Greiner, L. Seyfarth, T. Steinmetzer, O. Arad and S. Reissmann, *J. Prakt. Chem*., 2000, **342**, 537.
401. A.J. Souers, A. Rosenquist, E.M. Jarvie, M. Ladlow, W. Feniuk and J.A. Ellman, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2731.
402. L. Chen, K.J. Jensen, J. Tejbrant, J.E. Taylor, B.A. Morgan and G. Barany, *J. Pept. Res*., 2000, **55**, 81.
403. K. Gademann, M. Ernst, D. Seebach and D. Hoyer, *Helv. Chim. Acta*, 2000, **83**, 16.
404. Y. Tong, Y.M. Fobian, M. Wu, N.D. Boyd and K.D. Moeller, *J. Org. Chem*., 2000, **65**, 2484.
405. J. Liu, D.J. Underwood, M.A. Cascieri, S.P. Rohrer, L.-D. Cantin, G. Chicchi, A.B. Smith and R. Hirschmann, *J. Med. Chem*., 2000, **43**, 3827.
406. C. Péan, C. Créminon, A. Wijkhuisen, J. Grassi, P. Guenot, P. Jéhan, J.-P. Dalbiez, B. Perly and F. Djedaïni-Pilard, *J. Chem. Soc., Perkin. Trans. 2*, 2000, 853.
407. K. Nakamura, T.J. Baker and M. Goodman, *Org. Lett*., 2000, **2**, 2967.
408. I. Schon, O. Nyecki and A. Demeter, *J. Labelled Compd. Radiopharm*., 2000, **43**, 309.
409. U. Bhatt and G. Just, *Helv. Chim. Acta*, 2000, **83**, 722.
410. Y. Tong, J. Olczak, J. Zabrocki, M.C. Gershengorn, G.R. Marshall and K.D. Moeller, *Tetrahedron*, 2000, **56**, 9791.
411. V.V. Anokhina, I.V. Levushkina and E.I. Sorochinskaya, *Russ. J. Gen. Chem*., 1999, **69**, 1339.
412. B. Hargittai, N.A. Solé, D.R. Groebe, S.N. Abramson and G. Barany, *J. Med. Chem*., 2000, **43**, 4787.
413. T. Sasaki, K. Kobayashi, K. Minami, T. Kohno and K. Sato, *Pept. Sci*., 1999, 155.
414. X.-C. Wang and S.-P. Liang, *Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao*, 2000, **16**, 357.
415. E. Drakopoulou, K. Uray, G. Mezo, M.R. Price, C. Vita and F. Hudecz, *J. Pept. Sci*., 2000, **6**, 175.
416. K. Stromgaard, I. Bjornsdottir, K. Andersen, M.J. Brierley, S. Rizoli, N. Eldursi, I.R. Mellor, P.N.R. Usherwood, S.H. Hansen, P. Krogsgaard-Larsen and J.W. Jaroszewski, *Chirality*, 2000, **12**, 93.
417. S. Fiori, S. Pegoraro, S. Rudolph-Bohner, J. Cramer and L. Moroder, *Biopolymers*, 2000, **53**, 550.
418. J. Tilley, G. Kaplan, N. Fatouhi, B. Wolitzky and K. Rowan, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1163.
419. N. Fatouhi, P. Joshi, J.W. Tilley, K. Rowan, V. Schwinge and B. Wolitzsky, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1167.
420. N. Fatouhi, P. Joshi, D. Fry, C. Cook, J.W. Tilley, G. Kaplan, A. Hanglow, K. Rowan, V. Schwinge and B. Wolitzky, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1171.

421. A. Beck, N. Zorn, M.-C. Bussat, J.-F. Haeuw, N. Corcaïa, T.N. Nguyen, J.-Y. Bonnefoy and A. Van Dorsselaer, *J. Pept. Res.*, 2000, **55**, 24.
422. T.J. Deming and S.A. Curtin, *J. Amer. Chem. Soc.*, 2000, **122**, 5710.
423. A. Bentolila, I. Vlodavsky, R. Ishai-Michaeli, O. Kovalchuk, C. Haloun and A.J. Domb, *J. Med. Chem.*, 2000, **43**, 2591.
424. D.M. Lynn and R. Langer, *J. Amer. Chem. Soc.*, 2000, **122**, 10761.
425. A. Wierzbicki, C.A. Knight, T.J. Rutland, D.D. Muccio, B.S. Pybus and C.S. Sikes, *Biomacromolecules*, 2000, **1**, 268.
426. H. Tatehata, A. Mochizuki, T. Kawashima, S. Yamashita and H. Yamamoto, *J. Appl. Polym. Sci.*, 2000, **76**, 929.
427. N.L. Hungerford and G.W.J. Fleet, *J. Chem. Soc., Perkin Trans.* 1, 2000, 3680.
428. N.L. Hungerford, T.D.W. Claridge, M.P. Watterson, R.T. Aplin, A. Moreno and G.W.J. Fleet, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3666.
429. D.E.A. Brittain, M.P. Watterson, T.D.W. Claridge, M.D. Smith and G.W.J. Fleet, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3655.
430. J. Hwang and T.J. Deming, *Biomacromolecules*, 2001, **2**, 17.
431. J.M. Benns, J.-S. Choi, R.I. Mahato, J.-S. Park and S.W. Kim, *Bioconjugate Chem.*, 2000, **11**, 637.
432. C.-H. Deng, Y.-M. Li and Y.F. Zhao, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2000, **163**, 203.
433. Y. Fan, M. Kobayashi and H. Kise, *Polym. J.*, 2000, **32**, 817.
434. G. Radau and D. Rauh, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 779.
435. A.J. Harvey and A.D. Abell, *Tetrahedron*, 2000, **56**, 9763.
436. B.W. Clare, A. Scozzafava, F. Briganti, B. Iorga and C.T. Supuran, *J. Enzyme Inhib.*, 2000, **15**, 235.
437. D. Nöteberg, J. Brånalt, I. Kvanström, M. Linschoten, D. Musil, J.E. Nyström, G. Zuccarello and B. Samuelsson, *J. Med. Chem.*, 2000, **43**, 1705.
438. P.D. Edwards, R.C. Mauger, K. Cottrell, F.X. Morris, K.K. Pine, M.A. Sylvester, C.W. Scott and S.T. Furlong, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2291.
439. S. Hanessian, E. Balaux, D. Musil, L.-L. Olsson and I. Nilsson, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 243.
440. U. Baettig, L. Brown, D. Brundish, C. Dell, A. Furzer, S. Garman, D. Janus, P.D. Kane, G. Smith, C.V. Walker, X. Cockcroft, J. Ambler, A. Mitchelson, M.D. Talbot, M. Tweed and N. Wills, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1563.
441. C.K. Marlowe, U. Sinha, A.C. Gunn and R.M. Scarborough, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 13.
442. S.Y. Tamura, O.E. Levy, T.H. Uong, J.E. Reiner, E.A. Goldman, J.Z. Ho, C.R. Cohen, P.W. Bergum, R.F. Nutt, T.K. Brunck and J.E. Semple, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 745.
443. J. Cacciola, J.M. Fevig, P.F.W. Stouten, R.S. Alexander, R.M. Knabb and R.R. Wexler, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1253.
444. J.E. Semple, O.E. Levy, N.K. Minami, T.D. Owens and D.V. Siev, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2305.
445. S.Y. Tamura, M.I. Weinhouse, C.A. Roberts, E.A. Goldman, K. Masukawa, S.M. Anderson, C.R. Cohen, A.E. Bradbury, V.T. Bernardino, S.A. Dixon, M.G. Ma, T.G. Nolan and T.K. Brunck, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 983.
446. Y. Okada, Y. Matsumoto, Y. Tsuda, M. Tada, K. Wanaka, A. Hijikata-Okunomiya and S. Okamoto, *Chem. Pharm. Bull.*, 2000, **48**, 184.
447. I. Nakanishi, A. Fujikawa, K. Imai and A. Sato, *J. Pept. Res.*, 2000, **55**, 120.
448. Z.S. Li-Pan, H.V. Joshi and G.A. Digenis, *J. Enzyme Inhib.*, 1999, **15**, 63.

449. H.H. Wasserman, J.-H. Chen and M. Xia, *Helv. Chim. Acta*, 2000, **83**, 2607.
450. Y. Hayashi, K. Iijima, J. Katada and Y. Kiso, *Bioorg. Med. Chem. Lett*., 2000, **10**, 199.
451. G.S. Beligere and P.E. Dawson, *J. Amer. Chem. Soc*., 2000, **122**, 12079.
452. R.E. Morty, L. Troeberg, J.C. Powers, S. Ono, J.D. Lonsdale- Eccles and T.H.T. Coetzer, *Biochem. Pharmacol*., 2000, **60**, 1497.
453. K. Oscarsson, B. Classon, I. Kvarnstrom, A. Hallberg and B. Samuelsson, *Can. J. Chem*., 2000, **78**, 829.
454. J.D.A. Tyndall, R.C. Reid, D.P. Tyssen, D.K. Jardine, B. Todd, M. Passmore, D.R. March, L.K. Pattenden, D.A. Bergman, D. Alewood, S.-H. Hu, P.F. Alewood, C.J. Birch, J.L. Martin and D.P. Fairlie, *J. Med. Chem*., 2000, **43**, 3495.
455. T. Mimoto, N. Hattori, H. Takaku, S. Kisanuki, T. Fukazawa, K. Terashima, R. Kato, S. Nojima, S. Misawa, T. Ueno, J. Imai, K. Enomoto, S. Tanaka, H. Sakikawa, M. Shintani, H. Hayashi and Y. Kiso, *Chem. Pharm. Bull*., 2000, **48**, 1310.
456. A.D. Pivazyan, D.S. Matteson, L. Fabry-Asztalos, R.P. Singh, P.-f. Lin, W. Blair, K. Guo, B. Robinson and W.H. Prusoff, *Biochem. Pharmacol*., 2000, **60**, 927.
457. M.M. Sheha, N.M. Mahfouz, H.Y. Hassan, A.F. Youssef, T. Mimoto and Y. Kiso, *Eur. J. Med. Chem*., 2000, **35**, 887.
458. H. Matsumoto, T. Hamawaki, H. Ota, T. Kimura, T. Goto, K. Sano, Y. Hayashi and Y. Kiso, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1227.
459. N.J. Ede, S.N. Eagle, G. Wickham, A.M. Bray, B. Warne, K. Shoemaker and S. Rosenbaerg, *J. Pept. Sci*., 2000, **6**, 11.
460. E.S. Priestley and C.P. Decicco, *Org. Lett*., 2000, **2**, 3095.
461. M. Llinas-Brunet, M. Bailey, G. Fazal, E. Ghiro, V. Gorys, S. Goulet, T. Halmos, R. Maurice, M. Poirier, M.-A. Poupart, J. Rancourt, D. Thibeault, D. Wernic and D. Lamarre, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2267.
462. P.S. Dragovich, R. Zhou, S.E. Webber, T.J. Prins, A.K. Kwok, K. Okano, S.A. Fuhrman, L.S. Zalman, F.C. Maldonado, E.L. Brown, J.W. Meador, A.K. Patick, C.E. Ford, M.A. Brothers, S.L. Binford, D.A. Matthews, R.A. Ferre and S.T. Worland, *Bioorg. Med. Chem. Lett*., 2000, **10**, 45.
463. R. Joyeau, C. Maoulida, C. Guillet, F. Frappier, A.R.L. Teixeira, J. Schrevel, J. Santana and P. Grellier, *Eur. J. Med. Chem*., 2000, **35**, 257.
464. A. Stöckel-Maschek, C. Mrestani-Klaus, B. Stiebitz, H.-U. Demuth and K. Neubert, *Biochim. Biophys. Acta*, 2000, **1479**, 15.
465. N.C. Rockwell, D.J. Krysan and R.S. Fuller, *Anal. Biochem*., 2000, **280**, 201.
466. J.E. Sheppeck, H. Kar, L. Gosink, J.B. Wheatley, E. Gjerstad, S.M. Loftus, A.R. Zubiria and J.W.Janc, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2639.
467. M. Mergler, F. Dick, J. Gosteli and R. Nyfeler, *Lett. Pept. Sci*., 2000, **7**, 1.
468. K. Hojo, M. Maeda, S. Iguchi, T. Smith, H. Okamoto and K. Kawasaki, *Chem. Pharm. Bull*., 2000, **48**, 1740.
469. C. Charitos, C. Tzougraki and G. Kokotos, *J. Pept. Res*., 2000, **56**, 373.
470. W.R. Roush, A.A. Hernandez, J.H. McKerrow, P.M. Selzer, E. Hansell and J.C. Engel, *Tetrahedron*, 2000, **56**, 9747.
471. T. Schirmeister and M. Peric, *Bioorg. Med. Chem*., 2000, **8**, 1281.
472. I.O. Donkor, X. Zheng and D.D. Miller, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2497.
473. R. Tripathy, M.A. Ator and J.P. Mallamo, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2315.
474. L. Banfi, G. Guanti and R. Riva, *Chem. Commun*., 2000, 985.
475. Y. Nagao, S. Sano, K. Morimoto, H. Kakegawa, T. Takatani, M. Shiro and N. Katunuma, *Tetrahedron Lett*., 2000, **41**, 2419.
476. M. Pawelczak, K. Nowak and P. Kafarski, *Phosphorus, Sulfur Silicon Relat. Elem*.,

1998, **132**, 65.
477. J.F. Lynas, S.J. Hawthorne and B. Walker, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1771.
478. B. Walker, J.F. Lynas, M.A. Meighan and D. Brömme, *Biochem. Biophys. Res. Commun*., 2000, **275**, 401.
479. C. Pesenti, A. Arnone, A.M. Aubertin, P. Bravo, M. Frigerio, W. Panzeri, S. Schmidt, F. Viani and M. Zanda, *Tetrahedron Lett*., 2000, **41**, 7239.
480. G. Rosse, E. Kueng, M.G.P.Page, V. Schauer-Vukasinovic, T. Giller, H.-W. Lahm, P. Hunziker and D. Schlatter, *J. Comb. Chem*., 2000, **2**, 461.
481. X.-H. Tong and A. Hong, *Tetrahedron Lett*., 2000, **41**, 8857.
482. M. Lee and D.H. Kim, *Bioorg. Med. Chem*., 2000, **8**, 815.
483. A.A. Scozzafava and C.T.C.T. Superan, *Bioorg. Med. Chem*., 2000, **8**, 637.
484. A. Scozzafava and C.T. Supuran, *Bioorg. Med. Chem. Lett*., 2000, **10**, 499.
485. C.T. Supuran, F. Briganti, G. Mincione and A. Scozzafava, *J. Enzyme Inhib*., 2000, **15**, 111.
486. J.C.D. Müller, J. Ottl and L. Moroder, *Biochemistry*, 2000, **39**, 5111.
487. S.M. Dankwardt, R.J. Billedeau, L.K. Lawley, S.C. Abbott, R.L. Martin, C.S. Chan, H.E. Van Wart and K.A.M. Walker, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2513.
488. J.F. Lynas, S.L. Martin, B. Walker, A.D. Baxter, J. Bird, R. Bhogal, J.G. Montana and D.A. Owen, *Comb. Chem. High Throughput Screening*, 2000, **3**, 37.
489. J. Buchardt, C.B. Schiodt, C. Krog-Jensen, J.-M. Delaisse, N.T. Foged and M. Meldal, *J. Comb. Chem*., 2000, **2**, 624.
490. D.L. Steer, R.A. Lew, P. Perlmutter, A.I. Smith and M.-I. Aguilar, *J. Pept. Sci*., 2000, **6**, 470.
491. A. Scozzafava, M.A. Ilies, G. Manole and C.T. Supuran, *Eur. J. Pharm. Sci*., 2000, **11**, 69.
492. M.G. Natchus, R.G. Bookland, B. De, N.G. Almstead, S. Pikul, M.J. Janusz, S.A. Heitmeyer, E.B. Hookfin, L.C. Hsieh, M.E. Dowty, C.R. Dietsch, V.S. Patel, S.M. Garver, F. Gu, M.E. Pokross, G.E. Mieling, T.R. Baker, D.J. Foltz, S.X. Peng, D.M. Bornes, M.J. Strojnowski and Y.O. Taiwo, *J. Med. Chem*., 2000, **43**, 4948.
493. R.J. Davenport and R.J. Watson, *Tetrahedron Lett*., 2000, **41**, 7983.
494. M. North, *J. Pept. Sci*., 2000, **6**, 301.
495. J.A. Robl, L.M. Simpkins and M.M. Asaad, *Bioorg. Med. Chem. Lett*., 2000**, 10**, 257.
496. W. Han, Z. Hu, X. Jiang and C.P. Decicco, *Bioorg. Med. Chem. Lett*., 2000, **10**, 711.
497. G. Loidl, H.-J. Musiol, M. Groll, R. Huber and L. Moroder, *J. Pept. Sci*., 2000, **6**, 36.
498. H.S. Overkleeft, P.R. Bos, B.G. Hekking, E.J. Gordon, H.L. Ploegh and B.M. Kessler, *Tetrahedron Lett*., 2000, **41**, 6005.
499. C.E. O'Connell, C.A. Rowell, K. Ackermann, A.M. Garcia, M.D. Lewis and J.J. Kowalczyk, *Chem. Pharm. Bull*., 2000, **48**, 740.
500. E.K. Dolence, J.M. Dolence and C.D. Poulter, *J. Comb. Chem*., 2000, **2**, 522.
501. K.M. Huntington, T. Yi, Y. Wei and D. Pei, *Biochemistry*, 2000, **39**, 4543.
502. R.J. Cox, H. Jenkins, J.A. Schouten, R.A. Stentiford and K.A. Wareing, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2023.
503. H. Huang, P. Martasek, L.J. Roman and R.B. Silverman, *J. Med. Chem*., 2000, **43**, 2938.
504. L. Sautebin, L. Rombola, M. Di Rosa, G. Caliendo, E. Perisutti, P. Grieco, B. Severino and V. Santagada, *Eur. J. Med. Chem*., 2000, **35**, 727.
505. Q. Li, M. Moutiez, J.-B. Charbonnier, K. Vaudry. A. Ménez, E. Quéméneur and C. Dugave, *J. Med. Chem*., 2000, **43**, 1770.
506. K. Obi, J.-I. Uda, K. Iwase, O. Sugimoto, H. Ebisu and A. Matsuda, *Bioorg. Med. Chem. Lett*., 2000, **10,** 1451.

507. E.A. Garrard, E.C. Borman, B.N. Cook, E.J. Pike and D.G. Alberg, *Org. Lett*., 2000, **2**, 3639.
508. R. Andruszkiewicz, R. Jedrzejczak, T. Zieniawa, M. Wojciechowski and E. Borowski, *J. Enzym. Inhib*., 2000, **15**, 429.
509. D.L. Mohler, G. Shen and A.K. Dotse, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2239.
510. S. Matsumura, S. Sakamoto, A. Ueno and H. Mihara, *Chem.-Eur. J*., 2000, **6**, 1781.
511. M.A. Hossain, K. Hamasaki, H. Mihara and A. Ueno, *Chem. Lett*., 2000, 252.
512. M.A. Hossain, S. Matsumura, T. Kanai, K. Hamasaki, H. Mihara and A. Ueno, *J. Chem. Soc., Perkin Trans. 2*, 2000, 1527.
513. Y. Fukushima, *Recent Res. Dev. Pure Appl. Chem*., 1999, **3**, 21.
514. I. Obataya, S. Sakamoto, A. Ueno and H. Mihara, *Pept. Sci*., 1999, 361.
515. H. Kuribayashi, T. Takahashi, K. Nagata, A. Ueno and H. Mihara, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2227.
516. S. Matsumura, A. Ueno and H. Mihara, *Chem. Commun*., 2000, 1615.
517. Y. Takaoka, K. Suzuki and I. Fujii, *Pept. Sci*., 1999, 347.
518. J.D. Fisk, D.R. Powell and S.H. Gellman, *J. Amer. Chem. Soc*., 2000, **122**, 5443.
519. B. Baumeister and S. Matile, *Chem.-Eur. J*., 2000, **6**, 1739.
520. S. Kobayashi, H. Kobayashi, T. Yamaguchi, M. Nishida, K. Yamaguchi, M. Kurihara, N. Miyata and A. Tananka, *Chem. Pharm. Bull*., 2000, **48**, 920.
521. Y. Takahashi, T. Yamashita, A. Ueno and H. Mihara, *Tetrahedron*, 2000, **56**, 7011.
522. C. Das, V. Nayak, S. Raghothama and P. Balaram, *J. Pept. Res*., 2000, **56**, 307.
523. B.W. Gung, D. Zou and Y. Miyahara, *Tetrahedron*, 2000, **56**, 9739.
524. J.A. Robinson, *Synlett*, 2000, 429.
525. M. MacDonald, D.V. Velde and J. Aube, *Org. Lett*., 2000, **2**, 1653.
526. A.I. Jiménez, C. Cativiela, J. Gómez-Catalán, J.J. Pérez, A. Aubry, M. París and M. Marraud, *J. Amer. Chem. Soc*., 2000, **122**, 5811.
527. J.S. Davies, M. Stelmach-Diddams, R. Fromentin, A. Howells and R. Cotton, *J. Chem. Soc., Perkin Trans. 1*, 2000, 239.
528. H.-O. Kim, H. Nakanishi, M.S. Lee and M. Kahn, *Org. Lett*., 2000, **2**, 301.
529. M.A. Estiarte, M. Rubiralta, A. Diez, M. Thormann and E. Giralt, *J. Org. Chem*., 2000, **65**, 6992.
530. N. Carulla, C. Woodward and G. Barany, *Biochemistry*, 2000, **39**, 7927.
531. J.F. Espinosa and S.H. Gellman, *Angew. Chem., Int. Ed*., 2000, **39**, 2330.
532. A. Golebiowski, S.R. Klopfenstein, X. Shao, J.J. Chen, A.-O. Colson, A.L. Grieb and A.F. Russell, *Org. Lett*., 2000, **2**, 2615.
533. H.-J. Lee, I.-A. Ahn, S. Ro, K.-H. Choi, Y.-S. Choi, and K.- B. Lee, *J. Pept. Res*., 2000, **56**, 35.
534. A.J. Brouwer, H.J. van der Linden and R.M.J. Liskamp, *J. Org. Chem*., 2000, **65**, 1750.
535. N. Crisma, F. Formaggio, S. Mezzato, C. Toniolo, J. Savrda, J.-P. Mazaleyrat and M. Wakselman, *Lett. Pept. Sci*., 2000, **7**, 123.
536. A. Moretto, C. Peggion, F. Formaggio, M. Crisma, C. Toniolo, C. Piazza, B. Kaptein, Q.B. Broxterman, I. Ruiz, M.D. Diaz- de-Villegas, J.A. Galvez and C. Cativiela, *J. Pept. Res*., 2000, **56**, 283.
537. F. Formaggio, M. Crisma, P. Rossi, P. Scrimin, B. Kaptein, Q.B. Broxterman, J. Kamphuis and C. Toniolo, *Chem.-Eur. J*., 2000, **6**, 4498.
538. S.A. Hopkins, J.P. Konopelski, M.M. Olmstead and H.D. Banks, *Tetrahedron*, 2000, **56**, 9733.
539. T. Arai, T. Imachi, T. Kato and N. Nishino, *Bull. Chem. Soc. Jpn*., 2000, **73**, 439.
540. H. Zeng, R.S. Miller, R.A. Flowers and B. Gong, *J. Amer. Chem. Soc*., 2000, **122**,

2635.
541. C. Peggion, E. Mossel, F. Formaggio, M. Crisma, B. Kaptein, Q.B. Broxterman, J. Kamphuis and C. Toniolo, *J. Pept. Res.*, 2000, **55**, 262.
542. C. Didierjean, A. Aubry, F. Wyckaert and G. Boussard, *J. Pept. Res.*, 2000, **55**, 308.
543. M. Hattori, M. Oka, T. Hayashi and Y. Hirano, *Pept. Sci.*, 1999, 343.
544. M. Tanaka, N. Imawaka, M. Kurihara and H. Suemune, *Pept. Sci.*, 1999, 327.
545. T. Yamada, T. Ichino, R. Yanagihara and T. Miyazawa, *Pept. Sci.*, 1999, 303.
546. C. Peggion, R. Flammengo, E. Mossel, Q.B. Broxterman, B. Kaptein, J. Kamphuis, F. Formaggio, M. Crisma and C. Toniolo, *Tetrahedron*, 2000, **56**, 3589.
547. K.J. Jensen and G. Barany, *J. Pept. Res.*, 2000, **56**, 3.
548. J.V. Schreiber and D. Seebach, *Helv. Chim. Acta*, 2000, **83**, 3139.
549. X. Wang, J.F. Espinosa and S.H. Gellman, *J. Amer. Chem. Soc.*, 2000, **122**, 4821.
550. L.M. Likhosherstov, O.S. Novikova, A.O. Zheltova and V.N. Shibaev, *Russ. Chem. Bull.*, 2000, **49**, 1454.
551. B.G. Davis, M.A.T. Maughan, M.P. Green, A. Ullman and and J.B. Jones, *Tetrahedron Asymmetry*, 2000, **11**, 245.
552. Y. Ito, M. Gerz and Y. Nakahara, *Tetrahedron Lett.*, 2000, **41**, 1039.
553. S.P. Vincent, A. Schleyer and C.-H. Wong, *J. Org. Chem.*, 2000, **65**, 4440.
554. L. Jobron and G. Hummel, *Angew. Chem., Int. Ed.* , 2000, **39**, 1621.
555. I. Shin, H.-J. Jung and J.w. Cho, *Bull. Korean Chem. Soc.*, 2000, **21**, 845.
556. Z.-G. Wang, X. Zhang, D. Live and S.J. Danishefsky, *Angew. Chem., Int. Ed.*, 2000, **39**, 3652.
557. R.J. Tennant-Eyles, B.G. Davis and A.J. Fairbanks, *Tetrahedron: Asymmetry*, 2000, **11**, 231.
558. L. Jobron and G. Hummel, *Org. Lett.*, 2000, **2**, 2265.
559. K.M. Koeller, M.E.B. Smith and C.-H. Wong, *Bioorg. Med. Chem.*, 2000, **8**, 1017.
560. P. Sjölin and J. Kihlberg, *Tetrahedron Lett.*, 2000, **41**, 4435.
561. W.T. Jiaang, M.-Y. Chang, P.-H. Tseng and S.-T. Chen, *Tetrahedron Lett.*, 2000, **41**, 3127.
562. B. Holm, J. Broddefalk, S. Flodell, E. Wellner and J. Kihlberg, *Tetrahedron*, 2000, **56**, 1579.
563. N.B. Malkar, J.L. Lauer-Fields and G.B. Fields, *Tetrahedron Lett.*, 2000, **41**, 1137.
564. K. Haneda, H. Tanabe, M. Mizuno, T. Inazu, K. Tsumori, E. Munekata and K. Yamamoto, *Pept. Sci.*, 1999, 459.
565. J. Sander and H. Waldmann, *Chem.-Eur. J.*, 2000, **6**, 1564.
566. D. Bailey, D.V. Renouf, D.G. Large, C.D. Warren and E.F. Hounsell, *Carbohydr. Res.*, 2000, **324**, 242.
567. T.K. Chakraborty, S. Ghosh, S. Jayaprakash, J.A.R.P. Sharma, V. Ravikanth, P.V. Diwan, R. Nagaraj and A.C. Kunwar, *J. Org. Chem.*, 2000, **65**, 6441.
568. J.P. Malkinson, R.A. Falconer and I. Toth, *J. Org. Chem.*, 2000, **65**, 5249.
569. F. Peri, L. Cipolla, B.L. Ferla, P. Dumy and F. Nicotra, *Glycoconjugate J.*, 1999, **16**, 399.
570. I. Shin and J. Lee, *Synlett*, 2000, 1297.
571. T. Inazu, H.-K. Ishida, R. Nagano, K. Tanaka and K. Haneda, *Pept. Sci.*, 1999, 121.
572. F. Reichel, A.M. Roelofsen, H.P.M. Geurts, S.J. van der Gaast, M.C. Feiters and G.J. Boons, *J. Org. Chem.*, 2000, **65**, 3357.
573. P. Arya, K.M.K. Kutterer and A. Barkley, *J. Comb. Chem.*, 2000, **2**, 120.
574. K. Sadalapure and T.K. Lindhorst, *Angew. Chem., Int Ed.*, 2000, **39**, 2010.
575. Z.F. Wang and J.C. Xu, *Chin. Chem. Lett.*, 2000, **11**, 297.
576. G. Liu, S.-D. Zhang, S.-Q. Xia and Z.-K. Ding, *Bioorg. Med. Chem. Lett.*, 2000, **10**,

1361.
577. U. Urleb and S. Gobec, *Acta Pharm.* (*Zagreb*), 2000, **50**, 173.
578. K. Peilstöcker and H. Kunz, *Synlett*, 2000, 823.
579. K. Peilstöcker and H. Kunz, *Synlett*, 2000, 820.
580. W.-T. Jiaang, P.-H. Tseng and S.-T. Chen, *Synlett*, 2000, 797.
581. K.L. Halkes, P.M. St. Hilaire, A.M. Jansson, C.H. Godfredsen and M. Meldal, *J. Chem. Soc., Perkin Trans.* 1, 2000, 2127.
582. P.W. Glunz, S. Hintermann, L.J. Williams, J.B. Schwarz, S.D. Kuduk, V. Kudryashov, K.O. Lloyd and S.J. Danishefsky, *J. Amer. Chem. Soc.*, 2000, **122**, 7273.
583. K.M. Koeller, M.E.B. Smith, R.-F. Huang and C.-H. Wong, *J. Amer. Chem. Soc.*, 2000, **122**, 4241.
584. K. Ajisaka and M. Miyasato, *Biosci. Biotechnol. Biochem.*, 2000, **64**, 1743.
585. F. Sallas and S. Nishimura, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2091.
586. K. Kurokawa, H. Kumihara and H. Kondo, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1827.
587. S. Ando, Y. Nakahara, Y. Ito, T. Ogawa and Y. Nakahara, *Carbohydr. Res.*, 2000, **329**, 773.
588. S. Komba, O. Werdelin, T. Jensen and M. Meldal, *J. Pept. Sci.*, 2000, **6**, 585.
589. Y. Li, Y. Zhao and H. Waldmann, *Tsinghua Sci. Technol.*, 2000, **5**, 163.
590. Z. Kupihár, G. Váradi, E. Monostori and G.K. Tóth, *Tetrahedron Lett.*, 2000, **41**, 4457.
591. N. Kuder, T. Zelinski, T. Pathak, O. Seitz and H. Waldmann, *Bioorg. Med. Chem.*, 2000, **8**, 2433.
592. K. Miyoshi, A. Otaka, M. Kaneko, H. Tamamura and N. Fujii, *Chem. Pharm. Bull.*, 2000, **48**, 1230.
593. T. Kawakami, K. Hasegawa and S. Aimoto, *Bull. Chem. Soc. Jpn.*, 2000, **73**, 197.
594. W.-Q. Liu, M. Vidal, C. Mathé, C. Périgaud and C. Garbay, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 669.
595. P. Ruzza, A. Calderan, F. Cavaggion and G. Gianfranco, *Lett. Pept. Sci.*, 2000, **7**, 79.
596. S. Gobec and U. Urleb, *Phosphorus, Sulfur Silicon Relat.-Elem.*, 2000, **156**, 125.
597. D. Sikora and T. Gajda, *Tetrahedron*, 2000, **56**, 3755.
598. X.-J. Liu, R.-Y. Chen, L.-H. Weng and X.-B. Leng, *Heteroat. Chem.*, 2000, **11**, 422.
599. S. Chetyrkina, K. Estieu-Gionnet, G. Laïn, M. Bayle and G. Déléris, *Tetrahedron Lett.*, 2000, **41**, 1923.
600. J. Buchardt and M. Meldal, *J. Chem. Soc., Perkin Trans.* 1, 2000, 3306.
601. C.V. Walker, G. Caravatti, A.A. Denholm, J. Egerton, A. Faessler, P. Furet, C. García-Echeverría, B. Gay, E. Irving, K. Jones, A. Lambert, N.J. Press and J. Woods, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2343.
602. P. Furet, G. Caravatti, A.A. Denholm, A. Faessler, H. Fretz, C. García-Echeverría, B. Gay, E. Irving, N.J. Press, J. Rahuel, J. Schoepfer and C.V. Walker, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2337.
603. M.A. Kuprianova, M.N. Zhmak, D.O. Koroev, A.V. Chepurkin, O.M. Volpina and V.T. Ivanov, *Russ. J. Bioorg. Chem.*, 2000, **26**, 832.
604. G. Mezoe, N. Mihala, D. Andreu, and F. Hudecz, *Bioconjugate Chem.*, 2000, **11**, 484.
605. S. Cao, Z. Qin, M. Cai and Y. Shi, *Yaoxue Xuebao*, 1999, **34**, 368.
606. K.S. Kumar, V.N.R. Pillai and M.R. Das, *J. Pept. Res.*, 2000, **56**, 88.
607. M.-L. Valero, J.A. Camarero, T. Haack, M.G. Mateu, E. Domingo, E. Giralt and D. Andreu, *J. Mol. Recognit.*, 2000, **13**, 5.
608. I. Wirkus-Romanowska, H. Miecznikowska, A. Zablocka, K. Rybka, W. Fortuna,

R. Miedzybrodzki, S. Szymaniec, M. Janusz, J. Lisowski and G. Kupryszewski, *Pol. J. Chem.*, 2000, **74**, 219.
609. K. Uchida, K. Kato, K. Yamaguchi and H. Akita, *Heterocycles*, 2000, **53**, 2253.
610. Y. Wu and J.C. Xu, *Chin. Chem. Lett.*, 2000, **11**, 771.
611. M. Antopolsky and A Azhayev, *Tetrahedron Lett.*, 2000, **41**, 9113.
612. O. Seitz, *Angew. Chem., Int. Ed.*, 2000, **39**, 3249.
613. A. Stutz, C. Hobartner and S. Pitsch, *Helv. Chim. Acta*, 2000, **83**, 2477.
614. H. Narita, T. Moriguchi, K. Seio, M. Sekine, H. Miyaguchi, K. Sakamoto and S. Yokoyama, *Nucleosides, Nucleotides Nucleic Acids*, 2000, **19**, 1993.
615. T. Vilaivan, C. Khongdeesameor, P. Harnyuttanakorn, M.S. Westwell and G. Lowe, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2541.
616. A. Püschl, T. Tedeschi and P.E. Nielsen, *Org. Lett.*, 2000, **2**, 4161.
617. J.C. Verheijen, G.A. van der Marel, J.H. van Boom and N. Metzler-Nolte, *Bioconjugate Chem.*, 2000, **11**, 741.
618. X. Liu and S. Balasubramanian, *Tetrahedron Lett.*, 2000, **41**, 6153.
619. C.F. Bleczinski and C. Richert, *Org. Lett.*, 2000, **2**, 1697.
620. J.C. Verheijen, G.M. Grotenbreg, L.H. De Ruyter, P.A.M. Van der Klein, G.A. Van der Marel and J.H. Van Boom, *Tetrahedron Lett.*, 2000, **41**, 3991.
621. M. Planas, E. Bardají and G. Barany, *Tetrahedron Lett.*, 2000, **41**, 4097.
622. Z. Timár, L. Kovács, G. Kovács and Z. Schmél, *J. Chem. Soc., Perkin Trans. 1*, 2000, 19.
623. D. Gottschling, H. Seliger, G. Tarrason, J. Piulats, M. Wiersma and R. Eritja, *Lett. Pept. Sci.*, 2000, **7**, 35.
624. T. Takahashi, I. Kumagai, K. Hamasaki, A. Ueno and H. Mihara, *Pept. Sci.*, 1999, 397.
625. I. Kumagai, T. Takahashi, K. Hamasaki, A. Ueno and H. Mihara, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 377.
626. T. Takahashi, K. Hamasaki, I. Kumagai, A. Ueno and H. Mihara, *Chem. Commun.*, 2000, 349.
627. A. Sakakura and Y. Hayakawa, *Tetrahedron*, 2000, **56**, 4427.
628. P. Garner, S. Dey and Y. Huang, *J. Amer. Chem. Soc.*, 2000, **122**, 2405.
629. Z. Wang, L. Chen, S.F. Bayly and P.F. Torrence, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1357.
630. W. Maison, I. Schlemminger, O. Westerhoff and J. Martens, *Bioorg. Med. Chem.*, 2000, **8**, 1343.
631. H. Schwalbe, J. Wermuth, C. Richter, S. Szalma, A. Eschenmoser and G. Quinkert, *Helv. Chim. Acta*, 2000, **83**, 1079.
632. G. Karig, A. Fuchs, A. Büsing, T. Brandstetter, S. Scherer, J.W. Bats, A. Eschenmoser and G. Quinkert, *Helv. Chim. Acta*, 2000, **83,** 1049.
633. V. Marchán, C. Rodríguez-Tanty, M. Estrada, E. Pedroso and A. Grandas, *Eur. J. Org. Chem.*, 2000, 2495.
634. R. Schütz, M. Cantin, C. Roberts, B. Greiner, E. Uhlmann and C. Leumann, *Angew. Chem., Int. Ed.*, 2000, **39**, 1250.
635. D.A. Stetsenko and M.J. Gait, *J. Org. Chem.*, 2000, **65**, 4900.
636. Z. Miao and J.P. Tam, *Org. Lett.*, 2000, **2**, 3603.
637. N.M. Okeley, Y. Zhu and W.A. van der Donk, *Org. Lett.*, 2000, **2**, 3603.
638. Z. Kubica, T. Koźlecki and B. Rzeszotarska, *Chem. Pharm. Bull.*, 2000, **48**, 296.
639. A. Hammadi, H. Lam, M. Gondry, A. Ménez and R. Genet, *Tetrahedron*, 2000, **56**, 4473.
640. M. Royo, J.C. Jimenez, A. Lopez-Macia, E. Giralt and F. Albericio, *Eur. J. Org.*

Chem., 2001, 45.
641. H.-J. Kreuzfeld, C. Dobler, C. Fischer and W. Baumann, *Amino Acids*, 1999, **17**, 369.
642. K. Hojo, M. Maeda, S. Iguchi, H. Okamoto and K. Kawasaki, *Pept. Sci*., 1999, 135.
643. R.C.F. Jones and J. Dickson, *J. Pept. Sci*., 2000, **6**, 621.
644. T.J. Caulfield, S. Patel, J.M. Salvino, L. Leister and R. Labaudiniere, *J. Comb. Chem*., 2000, **2**, 600.
645. J. Van Ameijde and R.M.J. Liskamp, *Tetrahedron Lett*., 2000, **41**, 1103.
646. V.M.A. Felli, T.F. Martinelli and M.A.B. Da Silveira, *Rev. Bras. Cienc. Farm*., 1999, **35**, 47.
647. A.J. Brouwer, M.C.F. Monnee and R.M.J. Liskamp, *Synthesis*, 2000, 1579.
648. A. Calcagni, E. Gavuzzo, G. Lucente, F. Mazza, E. Morera, M.P. Paradisi and D. Rossi, *Biopolymers*, 2000, **54**, 379.
649. D.D. DesMarteau and V. Montanari, *Chem. Lett*., 2000, 1052.
650. A. Cheguillaume, I. Doubli-Bounoua, M. Baudy-Floch and P. Le Grel, *Synlett*, 2000, 331.
651. M. Ueki, S. Watanabe, R. Yamanaka, M. Ohta and O. Okunaka, *Pept. Sci*., 1999, 117.
652. M.C.F. Monnee, M.F. Marijne, A.J. Brouwer and R.M.J. Liskamp, *Tetrahedron Lett*., 2000, **41**, 7991.
653. N. Flaih, C. Gadjou, O. Lafont and H. Galons, *Synlett*, 2000, 896.
654. J. De Blas, E. Domínguez and J. Ezquerra, *Tetrahedron Lett*., 2000, **41**, 4567.
655. J.S. McMurray, O. Khabashesku, J.S. Birtwhistle and W. Wang, *Tetrahedron Lett*., 2000, **41**, 6555.
656. L. Wang and O. Phanstiel, *J. Org. Chem*., 2000, **65**, 1442.
657. K. Chavatte, J. Mertens and P. Van Den Winkel, *J. Labelled Compd. Radiopharm*., 2000, **43**, 1227.
658. P.G. Baraldi, R. Romagnoli, A. Duatti, C. Bolzati, A. Piffanelli, N. Bianchi, C. Mischiati and R. Gambari, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1397.
659. G.H. Van Domselaar, S.M. Okarvi, M. Fanta, M.R. Suresh and D.S. Wishart, *J. Labelled Compd. Radiopharm*., 2000, **43**, 1193.
660. J.L. Sutcliffe-Goulden, M.J. O'Doherty and S.S. Bansal, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1501.
661. T. Wakamiya, T. Yamashita, T. Fujii, Y. Yamaguchi, T. Nakano and M. Kirihata, *Pept. Sci*., 1999, 209.
662. B. Koksch, K. Mütze, S.N. Osipov, A.S. Golubev and K. Burger, *Tetrahedron Lett*., 2000, **41**, 3825.
663. M.C. Pirrung and K. Park, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2115.
664. M.S. Robillard, A.R.P.M. Valentijn, N.J. Meeuwenoord, G.A. Van der Marel, J.H. Van Boom and J. Reedijk, *Angew. Chem., Int. Ed*., 2000, **39**, 3096.
665. S.R. Gilbertson, S.E. Colibee and A. Agarkov, *J. Amer. Chem. Soc*., 2000, **122**, 6522.
666. A. Volonterio, P. Bravo and M. Zanda, *Org. Lett*., 2000, **2**, 1827.
667. A. Volonterio, P. Bravo, N. Moussier and M. Zanda, *Tetrahedron Lett*., 2000, **41**, 6517.
668. T. Yamada, Y. Urabe, R. Yanagihara and T. Miyazawa, *Pept. Sci*., 1999, 143.
669. T. Yamada, K. Makihaira, R. Yanagihara and T. Miyazawa, *Pept. Sci*., 1999, 259.
670. W. Maison, A. Lützen, M. Kosten, I. Schlemminger, O. Westerhoff, W. Saak and J. Martens, *J. Chem. Soc., Perkin Trans. 1*, 2000, 1867.
671. B. Alcaide, P. Almendros and C. Aragoncillo, *Chem. Commun*., 2000, 757.
672. L. Thévenet, R. Vanderesse, M. Marraud, C. Didierjean and A. Aubry, *Tetrahedron*

Lett., 2000, **41**, 2361.
673. R.C.F. Jones, S.J. Hollis and J.N. Iley, *Tetrahedron*: *Asymmetry*, 2000, **11**, 3273.
674. S.J. Bark, S. Schmid and K.M. Hahn, *J. Amer. Chem. Soc*., 2000, **122**, 3567.
675. R. Yanagihara, M. Katoh, M. Hanyuu, T. Miyazawa and T. Yamada, *J. Chem. Soc*., *Perkin Trans. 2*, 2000, 551.
676. R. Oliva, L. Falcigno, G. D'Auria, M. Saviano, L. Paolillo, G. Ansanelli and G. Zanotti, *Biopolymers*, 2000, **53**, 581.
677. A.A. Danilin, P.P. Purygin, N.V. Makarova, I.K. Moiseev, *Russ. J. Gen. Chem*., 1999, **69**, 1846.
678. M.J. Milewska, *Pol. J. Chem*., 2000, **74**, 447.
679. A. Gaucher, M. Wakselman, J.-P. Mazaleyrat, M. Crisma, F. Formaggio and C. Toniolo, *Tetrahedron*, 2000, **56**, 1715.
680. D. Seebach, A. Jacobi, M. Rueping, K. Gademann, M. Ernst and B. Jaun, *Helv. Chim. Acta*, 2000, **83**, 2115.
681. P. Heinonen, J. Rosenberg and H. Lönnberg, *Eur. J. Org. Chem*., 2000, 3647.
682. C.B. Carlson and P.A. Beal, *Org. Lett*., 2000, **2**, 1465.
683. C.B. Carlson and P.A. Beal, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1979.
684. I. Dufau and H. Mazarguil, *Tetrahedron Lett*., 2000, **41**, 6063.
685. T. Rühl, L. Hennig, Y. Hatanaka, K. Burger and P. Welzel, *Tetrahedron Lett*., 2000, **41**, 4555.
686. Y.F. Song, P. Yang and L. Sheh, *Chin. Chem. Lett*., 2000, **11**, 667.
687. Z.-R. Lu, S.-Q. Gao, P. Kopeckova and J. Kopecek, *Bioconjugate Chem*., 2000, **11**, 3.
688. S.S. Manfredini, M.M. Marastoni, R.R. Tomatis, E.E. Durini, S.S. Spisani, A.A. Pani, T.T. Marceddu, C.C. Musii, M.E.M.E. Marongiu and P.P. La Colla, *Bioorg. Med. Chem*., 2000, **8**, 539.
689. A. Bielawska, K. Bielawski and J. Palka, *Rocz. Akad. Med. Bialymstoku*, 1999, **44**, 190.
690. W.S. Meng, H.-H. Bui and I.S. Haworth, *Mol. Simul*, 2000, **24**, 215.
691. J.E. Oh and K.H. Lee, *Bioorg. Med. Chem*., 1999, **7**, 2985.
692. D.L. Boger and J.K. Lee, *J. Org. Chem*., 2000, **65**, 5996.
693. T. Kiyota, R. Yanagida, M. Oka, M. Miyoshi, S. Lee and G. Sugihara, *Bull. Chem. Soc. Jpn*., 2000, **73**, 2363.
694. S.-J. Zhang, W.-F. Xu, S.-C. Cheng and Y.-M. Yuan, *Zhongguo Yiyao Gongye Zazhi*, 2000, **31**, 440.
695. J.F. Sanz-Cervera, E.M. Stocking, T. Usui, H. Osada and R.M. Williams, *Bioorg. Med. Chem*., 2000, **8**, 2407.
696. A. Nefzi, M.A. Giulianotti and R.A. Houghten, *Tetrahedron*, 2000, **56**, 3319.
697. N. Schaschke, I. Assfalg-Machleidt, W. Machleidt, T. Laßleben, C.P. Sommerhoff and L. Moroder, *Bioorg. Med. Chem. Lett*., 2000, **10**, 677.
698. K.-Y. Tomizaki, H. Nishino, T. Kato, A. Miike and N. Nishino, *Chem. Lett*., 2000, 648.
699. M. Stanek and K. Grubmayr, *Monatsh. Chem*., 2000, **131**, 879.
700. H. Yamaguchi, H. Kodama, Y. Higashimoto, M. Jelokhani-Niaraki and M. Kondo, *Pept. Sci*., 1999, 447.
701. M. Onishi and H. Tamiaki, *Pept. Sci*., 1999, 357.
702. M. Takahashi, A. Ueno and H. Mihara, *Chem.-Eur. J*., 2000, **6**, 3196.
703. H. Morii, M. Ishimura and S. Honda, *Pept. Sci*., 1999, 323.
704. L.L. Klein, L. Li, H.-J. Chen, C.B. Curty, D.A. DeGoey, D.J. Grampovnik, C.L. Leone, S.A. Thomas, C.M. Yeung, K.W. Funk, V. Kishore, E.O. Lundell, D.

Wodka, J.A. Meulbroek, J.D. Alder, A.M. Nilius, P.A. Lartey and J.J. Plattner, *Bioorg. Med. Chem.*, 2000, **8**, 1677.
705. B. Kundu, S.K. Rastogi, S. Batra, S.K. Raghuwanshi and P.K. Shukla, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1779.
706. J. Xiao, G. Yuan, W. Huang, A.S.C. Chan and K.-L. D. Lee, *J. Org. Chem.*, 2000, **65**, 5506.
707. M.M. Rowshon Alam, M.M. Maeda and S.S. Sasaki, *Bioorg. Med. Chem.*, 2000, **8**, 465.
708. R.C. Lasey, S.S. Banerji and M.Y. Ogawa, *Inorg. Chim. Acta*, 2000, **300-302**, 822.
709. A. Dudkowiak, T. Kusumi, C. Nakamura and J. Miyake, *J. Photochem. Photobiol. A*, 1999, **129**, 51.
710. D.-G. Liu, Z.-J. Yao, Y. Gao and T.R. Burke, *Org. Prep. Proced. Int.*, 2000, **32**, 197.
711. A. Datta and G. Veeresa, *J. Org. Chem.*, 2000, **65**, 7609.
712. J.P. Tam, Y.-A. Lu and J.-L. Yang, *Biochemistry*, 2000, **39**, 7159.
713. C. Schumann, L. Seyfarth, G. Greiner and S. Reissmann, *J. Pept. Res.*, 2000, **55**, 428.
714. K.-H. Lee and J.-E. Oh, *Bioorg. Med. Chem.*, 2000, **8**, 833.
715. K. Ryan, L.J. Gershell and W.C. Still, *Tetrahedron*, 2000, **56**, 3309.
716. E. Kawanishi, K. Higuchi and A. Ishida, *Heterocycles*, 2000, **52**, 425.
717. C. Douat, A. Heitz, J. Martinez and J.A. Fehrentz, *Tetrahedron Lett.*, 2000, **41**, 37.
718. M. Groarke, B. Hartzoulakis, M.A. McKervey, B. Walker and C.H. Williams, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 153.
719. A.Y. Kornilova, J.F. Wishart, W. Xiao, R.C. Lasey, A. Fedorova, Y.-K. Shin and M.Y. Ogawa, *J. Amer. Chem. Soc.*, 2000, **122**, 7999.
720. M. Hackel, H.-J. Hinz and G.R. Hedwig, *Phys. Chem. Chem. Phys.*, 2000, **2**, 4843.
721. T. Yamazaki, K. Nakagomi, Y. Sadakane, T. Tanimura and T. Akizawa, *Chromatography*, 1999, **20**, 312.
722. F. Moffatt, P. Senkans and D. Ricketts, *J. Chromatogr.*, 2000, **891**, 235.
723. D.L. Husband, C.T. Mant and R.S. Hodges, *J. Chromatogr., A*, 2000, **893**, 81.
724. V. Sanz-Nebot, F. Benavente and J. Barbosa, *J. Chromatogr. A*, 2000, **870**, 315.
725. K. Walhagen, K.K. Unger and M.T.W. Hearn, *J. Chromatogr.*, 2000, **893**, 401.
726. Y. Zhang and F.A. Gomez, *Electrophoresis*, 2000, **21**, 3305.
727. N. Tsibakhashvili, V. Barnov, K. Matiashvili and L. Mosulishvili, *Izv. Akad. Nauk Gruz., Ser. Khim.*, 1999, **25**, 237.

3

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

BY JOHN S. DAVIES

1 Introduction

A change of authorship to this chapter this year has not altered the basic structure from that found in last year's chapter in Volume 32.[1] Papers covering the year 2000 have been sourced from CA Selects[2] on Amino Acids Peptides and Proteins (up to Issue 12, June 2001) and an increasing dependence on the Web of Science databases[3] on the Internet. Regular readers might detect a slight change of emphasis in the content of parts of the chapter, as a result of the change of authorship. Overlap on the cyclic peptide content with Chapter 4 has been reduced, leaving the latter Chapter as the more comprehensive coverage of that area. Due to lack of access to the more pharmacological journals, this aspect is less emphasised in the current report, although whenever a good abstract was available every attempt has been made to summarise the highlights.

Publications emanating from conference proceedings have not been searched and patents have not been used as source material. Due to limitation of space, whenever original works involve reports on a large number of analogues, only the more potent examples have been included.

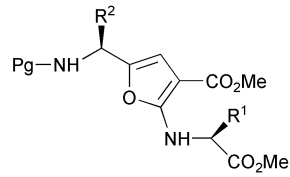
2 Peptide Backbone Modifications and Peptide Mimetics

Much of the subject material discussed under this section has been the subject of a monograph[4] on Peptidomimetic Protocols. Individual chapters within the monograph are referred to later under the appropriate sub-division. A complete issue of Tetrahedron Symposia in Print[5] has been dedicated to recent advances in peptidomimetics. However, once the isostere has been constructed, it still needs to be incorporated into a sequence, so it is timely to receive an update[6] of the bibliography pertaining to chemical synthesis of peptides.

2.1 Aza, Oxazole, Oxadiazole, Triazole and Tetrazole Peptides. – Solid phase synthesis of azapeptides is inclined to give chain-terminated hydantoin derivatives, due to intramolecular nucleophilic attack on an activated intermediate. A

Amino Acids, Peptides and Proteins, Volume 33

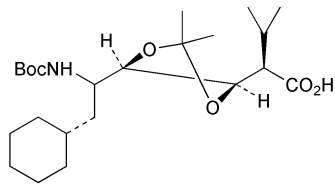
protocol has now been developed[7] which utilises *N*-2-hydroxy-4-methoxy benzyl (Hmb) as a reversible amide bond protecting group. Hydantoin-free azapeptides are produced by this route. A family of chiral oxazoles (**1a–d**) have been synthesised[8] in multigram quantities, for insertion into peptides. Ease of insertion has been demonstrated by the preparation of an endothelin-1 antagonist, based on the C-terminal hexapeptide of the endothelin-1 receptor. 3-Acylaminoalkyl-5-alkoxycarbonyl-1,2,4-triazoles have been synthesised[9] for insertion into an Arg-Gly-Asp motif, and 1,2,4-triazoles, 1,2,4- and 1,3,4-oxadiazoles have been the subject of a 46-reference review[10] of their use as isosteric replacement of peptide bonds. The 1,5-disubstituted tetrazole (Ψ [CN_4]) has been similarly reviewed[11] as *cis*-amide bond surrogates.



	R^1	R^2	Pg
(1) a	i-Pr	CH_2Ph	Z
b	s-Bu	CH_2COOBn	Boc
c	CH_2Ph	Me	Z
d	Me	i-Bu	Z

2.2 Ψ[*E*-CH=CH], Ψ[$CON^-N^+R^1R^2$], Ψ[dihydroxyethylene], Ψ[hydroxyethylene], Ψ[CHOH-cyclopropyl-CONH], Ψ[CH_2O], Ψ[CF=C], Ψ[NHCH(CF_3)], Ψ[CH_2N(COR)], Ψ[NHCO] and Ψ[SO_2NH]. – Cuprate opening of vinyl aziridines has been reviewed[12] as a versatile route to *E*-alkene isosteres, while aminimides bearing the isostere Ψ [$CON^-N^+R^1R^2$] have been discussed[13] as novel peptidomimetic units. The synthesis of (**2**) is seen[14] as providing a dihydroxyethylene isostere for peptidomimetic work, while two α-trifluoromethyl-amino-β-hydroxypeptide isosteres have been inserted[15] to create a pepstatin analogue, which did not yield any antiviral or cytotoxic activity. The substitution of Ψ [CHOH-cyclopropyl-CONH] as a replacement for the Gly^2-Gly^3 subunit of Leu-enkephalin has been reviewed[16] in 17 references, and synthetic procedures leading to the dipeptide isosteres Phe-Ψ [CH_2O]-spiro C_x and Phe-Ψ [CH_2O]-allylglycine have been recorded.[17] Fluoro-olefine isosteres retain the fixed conformation of Ψ [CH=CH], but probably more accurately mimic the electronic features of the amide bond, in its dipole moment, charge distribution and electrostatic potential.[18] Incorporation of Ψ[NHCH(CF_3)] as a surrogate of retro- and retro-inverso amides in solid phase protocols has been announced.[19] The procedures have been adapted for application to the production of protease inhibitor libraries.

A new strategy[20] for synthesising backbone cyclic peptides involves *N*-functionalisation to yield mimetic units capable of elaboration of *N*-amino and *N*-carboxy functions. The general structural unit synthesised is Fmoc-AA-Ψ[$CH_2N(CO(CH_2)_n)$-X], which allows the use of a great variety of ω-amino and

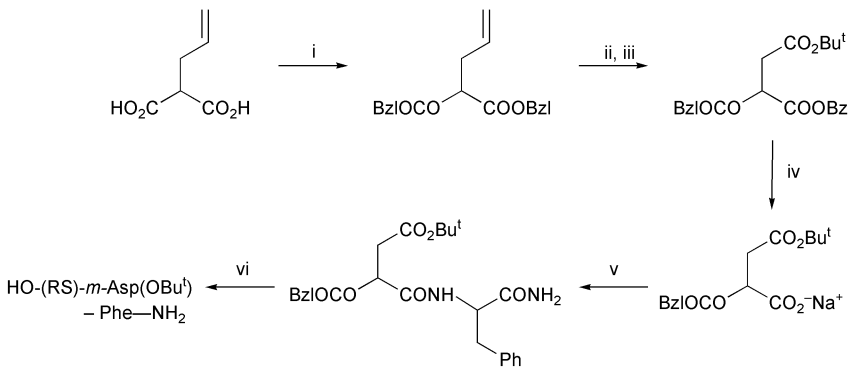


(2)

Fmoc-D-Pheψ[CH_2—N]Phe–OH
$CO(CH_2)_2R$

(3) R = NHAlloc
(4) R = COOAll

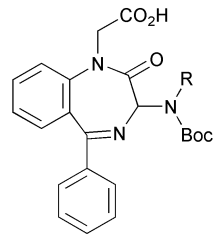
α,ω-dicarboxylic acids differing in chain lengths, as exemplified by (**3**) and (**4**). Backbone cyclic peptide analogues of bradykinin, containing N- or C-terminal arginine have been prepared[21] using such procedures. Introduction of retro inverso peptide bonds into linear RGD peptides has necessitated[22] a novel scheme (**Scheme 1**) for the introduction of a malonyl aspartyl residue into the peptide chain. The natural amino sulfonic acid, taurine (Tau), is the source of $\Psi[SO_2NH]$ in *Z*-Tau-Pro-D-Phe-NH-Pr^i which has been specifically designed and synthesised to test the ability of the sulfonamido group to participate in a type II β-turn. However, β-turn structure is not seen in the crystal and has only a minor contribution to make in $CDCl_3$ solution.[23] Foldamers, a class of β-peptides which have been shown to fold into defined 3D-structures similar to natural peptides, have had their peptide bonds replaced by sulfonamide groups.[24] The isostere replacement was achieved due to the availability of Fmoc-AaaΨ $[CH_2SO_2]$-Cl, where Aaa represents, Ala, Val or Leu, but it is not clear yet whether the isostere has altered the conformation. The application of 3-amino-1-carboxymethylbenzodiazepine (**5**) as a conformationally-constrained dipeptide mimic has been reviewed.[25] Three β-thiopeptide analogues of H-(β-HVal-β-HAla-β-Hleu)$_2$-OH with one, two and three C=S groups in the N-terminal positions have been syntheised[26] selectively using Lawesson's reagent. The peptide containing three C=S groups was more soluble in $CHCl_3$ than its oxygen analogue and showed a M-3_{14}-helical structure similar to the non-thionated analogue.



Reagents : i, BzlOH; ii, $KMnO_4$; iii, isobutene; iv, OH^-; v, H—Phe—NH_2/DCC/HOAt; vi, H_2/Pd/C

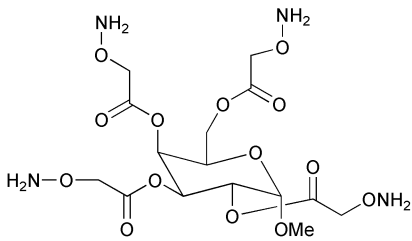
Scheme 1

2.3 Rigid Amino Acid, Peptide and Turn Mimetics. – Synthesis and conformational studies on novel templates used in the construction of β-hairpin mimetics

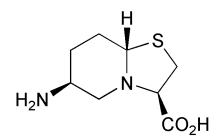


(5)

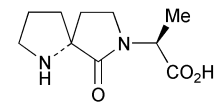
have been reviewed[27] in the context of drug and vaccine discovery. The role of peptidomimetics as inhibitors of platelet aggregation and their possible application as anti-thrombotic agents has been reviewed.[28] The preparation of polyhydroxy-pyrrolidines, piperidines and azepans from D-mannitol has been discussed[29] in the context of the development of non-peptide mimics of somatostatin and sandostatin. An aminoxy-functionalised D-galactose template (**6**) can function as a scaffold for the *de novo* design of proteins, by chemical ligation of peptide aldehydes onto the aminoxy groups.[30]



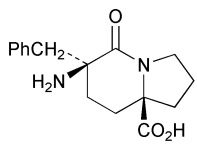
(6)



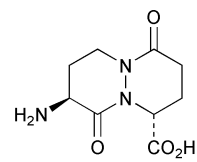
(7)



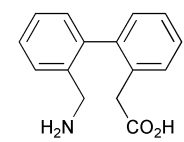
(8)



(9)



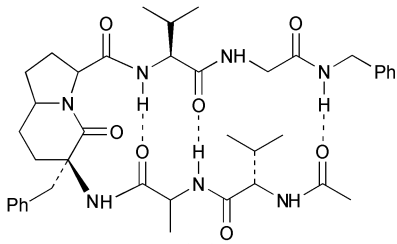
(10)



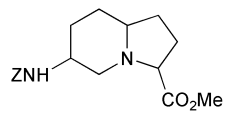
(11)

Some well-known β-turn mimetics (**7–11**) have been scrutinised[31] for their true ability to mimic the β-turn structure in a cyclic hexapeptide. Molecular dynamics and stochastic molecular mechanics simulation procedures were used to rank the mimetics and in terms of turn-induction, the most popular example (**7**) had to be content with 3rd place. The pecking order in turn-induction ability was (**9**) > (**8**) > (**7**) > (**10**) > (**11**). The authors suggest that all potential turn mimetics should be scrutinised by simulation using these computing methods. Bicyclic lactams not very different from (**8**) have been analysed[32] for their folding characteristics within the acylated hexapeptide such as (**12**). Type II′ compact β-turns were the main characteristics detected by NMR and IR spectroscopy, and computer modelling. In connection with a programme directed towards the synthesis of constrained GnRH analogues, all eight diastereoisomers of (**13**) have been synthesised[33] in gram quantities, from readily available (*R*) and (*S*) pyroglutamine

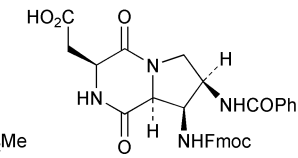
esters. The template molecule (**14**) derived from aspartic acid and 2*S*,3*R*,4*R*-diaminoproline, when inserted[34] into cyclo(Ala-Asn-Pro-Asn-Ala-Ala-template), gave evidence, in its NMR spectra, of a β-hairpin conformation.



(12)

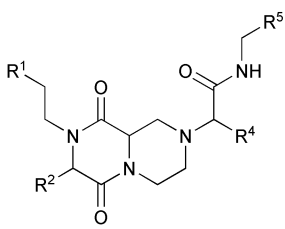


(13)

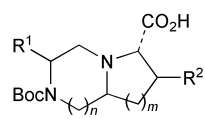


(14)

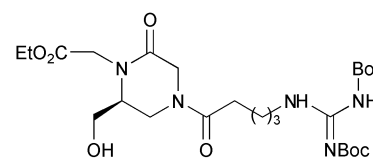
A Merrifield resin-bound piperazine -2-carboxylic acid, when subjected to the Petasis reaction, has been shown [35] to be an efficient source of the β-turn mimetic (**15**). Bicyclic piperazinones such as (**16**) have been examined[36] as conformational restrictors, while chiral piperazinones can be generated readily[37] from D-glucosamine and amino acid esters. An example of their use to conformationally restrict an RGD motif is shown in (**17**). The potential β-turn mimetic (**18**) has been produced[38] *via* an on-resin Ugi reaction protocol, starting with the resin ester of *N*-Boc-diaminopropanoic acid, α-bromo acid, an aldehyde and an isocyanide. Two separate research groups have probed for the optimum affinity between thyrotropin releasing hormone (TRH) and its receptor, by using peptidomimetic units, but neither the piperazine-2-one analogue (**19**)[39] nor the *cis*-peptide analogues (**20**) and (**21**)[40] showed any agonist or antagonist behaviour. γ-Lactam bridges have been the subject of a short review[41] and both the *cis*-form (3*R*,8*R* isomer)[42] and the *trans*-form (3*S*,8*R*-isomer)[43] of the eight-membered lactam (**22**) have been synthesised. The *cis* disubstituted form exists in a semi-extended conformation and exhibits head to tail self-recognition (K_{dim} 100



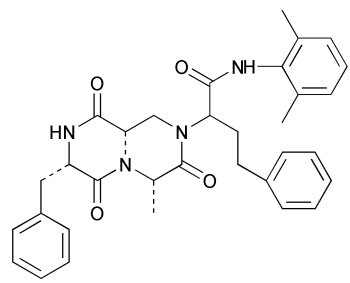
(15)



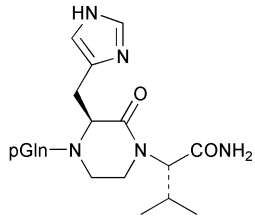
(16)



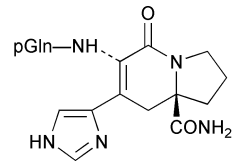
(17)



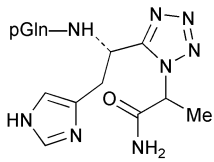
(18)



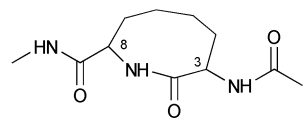
(19)



(20)



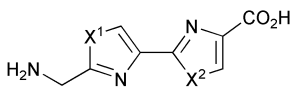
(21)



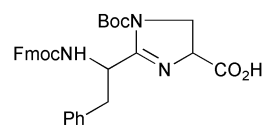
(22)

$\pm$ 20 $dm^3\,mol^{-1}$), while the *trans*-form shows a classical type VI β-turn in polar solutions.

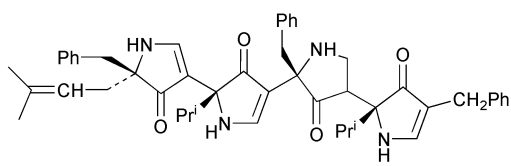
Tripeptide mimetics (**23a–c**) have been investigated as their Boc derivatives in the solid state[44] and following *ab initio* calculations it is found that the *anti* conformation is 20 kJ mol^{-1} more stable for (**23 a** and **b**) but only 4 kJ mol^{-1} for (**23c**). Two diastereoisomers of the imidazoline dipeptide mimetic (**24**) have been synthesised[45] while a theoretically predicted β-turn conformation for a D,L alternating polypyrrolinone has been confirmed by the synthesis and NMR investigations on (**25**).[46] Constraining amino acid side chains using cyclopropane rings is now well known[47] and has the advantage of allowing four different side chain orientations. The highly constrained cyclopropane analogue (**26**) has been synthesised[48] in two chirally pure forms. The (2*S*,3*S*) diphenyl form adopts a classical β-II turn, while the (2*R*,3*R*)-form exhibits an open β-II turn which lacks the *i* to *I* + 3 H-bond with a γ-turn centred at the cyclopropane unit. The phosphotyrosine unit of the well known SH2 antagonist Ac-pTyr-Glu-Glu-Ile-OH has been constrained[49] *via* the analogue (**27**) and binding studies show that the cyclopropane ring seems to correctly mimic the bound orientation of the phosphotyrosine moiety. The constraining of aromatic acids *via* larger poly-



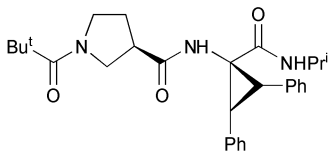
(23) a X^1 = S, X^2 = S
b X^1 = O, X^2 = S
c X^1 = S, X^2 = O



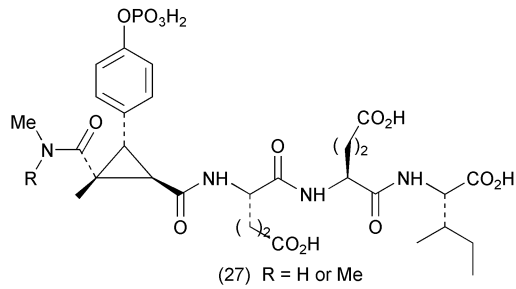
(24)



(25)



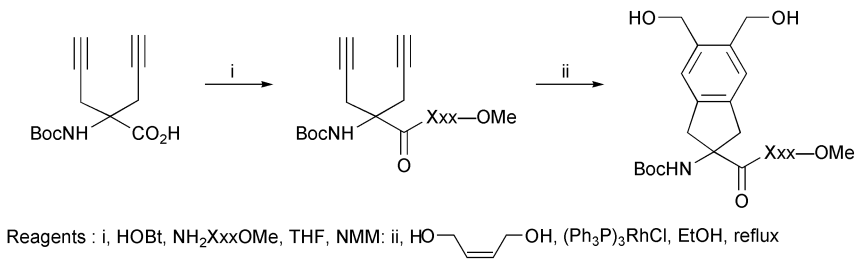
(26)



(27) R = H or Me

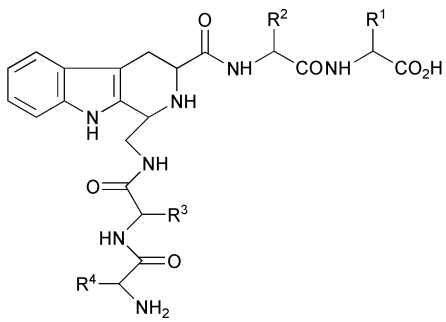
methylene rings has been reviewed[50] and a novel strategy for incorporating a constrained phenylalanyl unit has been evolved[51]as summarised in **Scheme 2**. The tetrahydro-β-carboline unit in (**28**) was constructed[52] *via* a Pictet–Spengler condensation of a resin bound Trp-containing fragment with a Fmoc-amino aldehyde.

The involvement of α,α-disubstituted amino acids and bicyclic lactams as peptidomimetic building blocks are the subject of a short review[53] while the novel spirocyclic azirinyl dipeptide synthon (**29**) has been used[54] in the successful synthesis of the analogue (**30**) of the C-terminal nonapeptide of the antibiotic

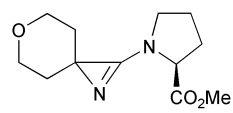


Reagents : i, HOBt, $NH_2XxxOMe$, THF, NMM: ii, HO—⟨—⟩—OH, $(Ph_3P)_3RhCl$, EtOH, reflux

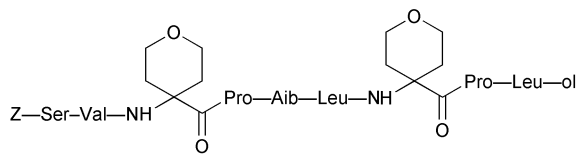
Scheme 2



(28)

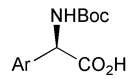


(29)

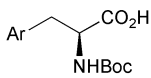


(30)

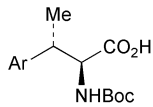
trichovirin 1. The largest $C_\alpha C_\alpha$-disubstituted glycine reported up to now has been synthesised[55] from 1-aminocyclododecane-1-carboxylic acid and inserted into model peptides using solution phase techniques. X-ray data confirmed that β-bends and 3_{10} helices were preferentially adopted by peptides containing these residues. 2,4,6-Trimethylphenyl (mesityl) groups substituted in the side chains of amino acids impose conformational rigidity and increased lipophilicity on amino acid residues. This information has been accumulated[56] from NMR data of enantiometrically pure forms of (**31–33**).



(31) Ar = mesityl



(32) Ar = mesityl

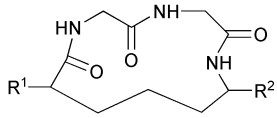


(33) Ar = mesityl

3 Cyclic Peptides

As was explained in the introduction to this chapter, the comprehensive coverage of this topic can now be seen in Chapter 4 of this volume. However, this chapter is not denuded of all references to conformational constraining *via* cyclisation, as many of the analogues of biologically active peptides discussed in the next section are examples of cyclic peptides. However, a few examples are more at home here as a logical extension of Section 2 or as examples of specific techniques in their construction.

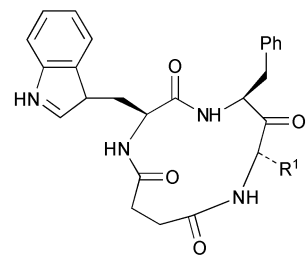
Constraining[57] of Gly-Gly residues by 6-aminocaproic acid has given the β-turn mimetics (**34**) and (**35**), while in a search[58] for lower molecular weight compounds with good antagonistic potency at the human tachykinin NK-2 receptor, cyclic peptides typified by (**36**) were synthesised. The best lead was the *R*-form of (**36**) $R^1 = PhCH_2$ which had a $pK_i = 8.7 \pm 0.3$. A β-hexapeptide has been clamped[59] into a 3_{14}-helix conformation through the synthesis of (**37**). A cyclo-release reaction[60] from solid phase resin, triggered by a dimethyl(thio)sulfonium moiety, has given rise to a series of peptides such as (**38**) in yields ranging from 30–80%. A thioether link, reminiscent of the naturally



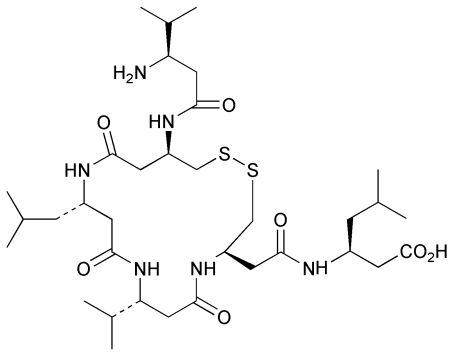
(34) R^1 = H, R^2 = 

(35) R^1 = 

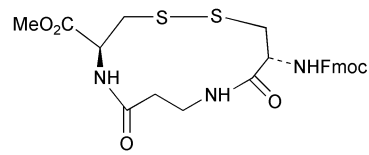
, R^2 = H



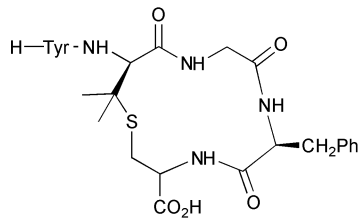
(36)



(37)



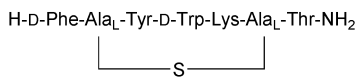
(38)



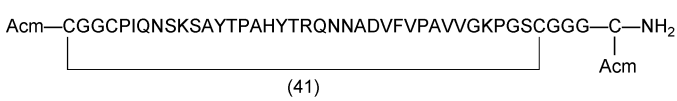
(39)

occurring lantibiotics has been incorporated[61] into a bridged opioid (**39**). It is highly δ-receptor selective, while an amine-bridged analogue, H-Tyr-cyclo[N_{γ}Me)-D-A_2bu-Gly-Phe-$NHCH_2CH_2$], though non-selective, is one of the most potent opioids prepared. The sandostatin analogue TT-232 [D-Phe-c(Cys-Tyr-D-Trp-Lys-Cys)-Thr-NH_2] has seen its disulfide ring being replaced[62] by a thioether bridge giving (**40**). The analogue adopts very similar conformations to TT-232 in solution, and results indicate that anti-tumour activity is retained.

Peptides such as (**41**) representing the surface loop 4 of class 1 Outer Membrane Protein (OMP) of *Neisseria meningitidis* have been synthesised[63] using Boc/Bzl protocols with the Mob protecting group being used to selectively generate the disulfide ring in the presence of cysteines protected by Acm. After removal of the Acm groups in (**41**), polymeric forms of the peptide were produced, and these elicited antisera which showed opsonic activity but no bactericidal activity. The regioselective formation of the two disulfide bonds of α-conotoxin SI (**42**) has been carried out using a one-pot procedure.[64] The first disulfide bond was formed by simultaneous cleavage and oxidation of t-butyl groups in TFA/DMSO at room temperature, while subsequent heating of this

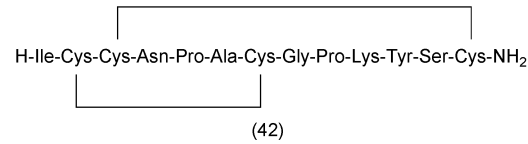


(40)

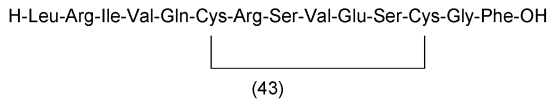


(41)

solution cleaved the 4-methyl benzyl protecting groups with subsequent oxidation to give (**42**). The anti-obesity peptide (**43**) has undergone[65] biological and



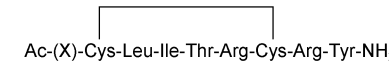
(42)



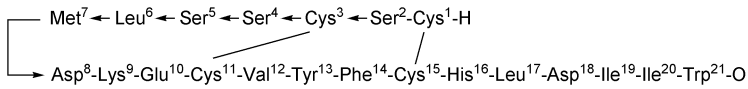
(43)

conformational analysis. Representing the 177–191 region of human growth hormone, it was shown to stimulate lipolysis and inhibition of lipogenesis *in vitro*. NMR studies showed that the cyclic region of (**43**) adopts type I β-turns at Ser^{8}-Val^{9}, Glu^{10}-Gly^{11} and Ser^{12}-Cys^{13}-Gly^{14}-Phe^{15}, and when compared to the X-ray crystal structure of the same residues in the human growth hormone, some structural similarity has been retained. The result[66] of the synthesis and testing of peptides representing the C-terminal sequence of neuropeptide Y shows that the analogues YM 42454 (**44**) and (**45**) have high binding at the Y_1-receptor. A novel heparin-binding neurotrophic factor consisting of 136 residues and five intramolecular disulfide bonds has been synthesised[67] in the solution phase using Boc-protocols, coupling being carried out in solution using EDC/HOOBt. Acm was used to protect the cysteines, and after removal with $Hg(OAc)_2$, the deprotected peptide underwent an oxidative folding reaction, assembling the disulfide bridges in their natural positions. Fragment peptides were also tested for their biological activities and it was confirmed that the C-terminal half 65-136 is responsible for the expression of biological activity in the same manner as human midkine, another heparin-binding neurotrophic growth factor. Endothelin-1 (**46**) and a 1-16 fragment of the receptor of the plasminogen activator 1, both containing Met together with Cys residues, could be successfully prepared[68] by cyclisation of the free SH groups with hydrogen peroxide. The Met to Met-oxide oxidation was suppressed by working at a pH optimum of 8.5 to 9. Four different strategies[69] have been compared for the formation of the two disulfide bridges in the recently discovered food intake stimulator, orexin A (hypocretin1), (**47**). 'Best of the bunch' was a one-step cyclisation in solution, based on I_2/AcOH oxidation, after selective protection by S-Acm and S-trityl groups. A yield of 35% was recorded in this protocol, while unselective oxidation gave a mixture of four possible isomers. On-resin cyclisation was the most elegant, but only produced 10% yield.

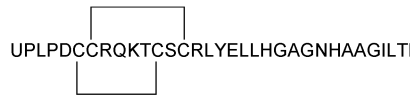
The essential strategy for the synthesis of medium-sized cyclic dehydropeptides, based on experiences with alternariolide (AM-toxin I) has been reviewed.[70] Cyclic analogues of the fragment Tyr-Val-Pro-Leu-Phe-Pro from proline rich protein (PRP) have been synthesised by solid phase techniques.[71] Conformational studies showed that disulfide bridged analogues were the most rigid.



(44) X = D
(45) X = L



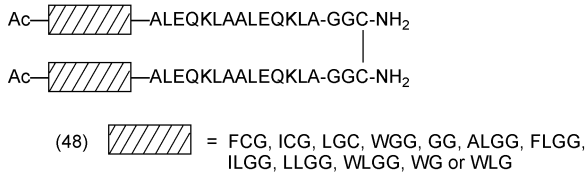
(46)



(47)

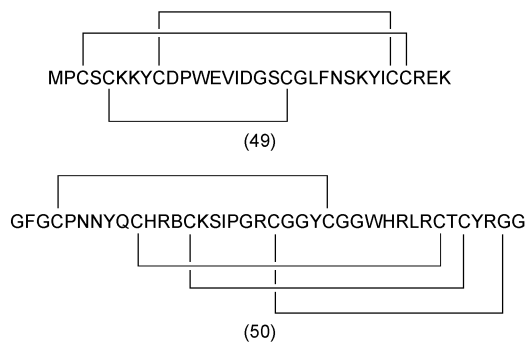
4 Biologically Active Peptides

4.1 Peptides Involved in Alzheimer's Disease. – The cellular and molecular basis of β-amyloid precursor protein (β-APP) has been reviewed.[72] Emphasis is made of the regulation of β-APP cleavage through diverse signal transduction mechanisms. The β-amyloid (Aβ), a 39–43 residue peptide, which is the proteolytic product of the membrane bound protein, is the main component of amyloid plaques found in brain lesions of patients suffering from Alzheimer's disease. Its N-terminal sequence up to residue 28 is deemed to be hydrophilic and the fragment (12–28) of this region has been used[73] to test for the early stages of aggregation. NMR and CD spectroscopic studies on millimolar solutions close to the isoelectric point show that high ionic strength and high temperature promotes aggregation and β-sheet formation. Overall it was felt that early stages in fibrillogenesis were being mimicked under these conditions. A sensitive immunoassay using Ciphergen's Seldi™ protein chip system has been developed[74] to measure all the variant Aβ in culture supernatants, which will be useful in screening inhibitors of the proteases involved. Using the assay it has been shown that increasing intracellular cholesterol increases the activities of both β- and γ-secretase, and increased α-secretase cleavage occurs by changing the intracellular targetting of amyloid precursor glycoprotein. A dimeric peptide (**48**), has been designed[75] to monitor the structural transition from α-helix to β-sheet and self assembly into amyloid fibrils. Peptide (**48**) initially forms a coiled-coil α-helix and the self initiated transition to β-sheet was induced by appropriate hydrophobic residues being attached at the N-terminus. In the β-sheet form the peptides self-assembled into the amyloid.



4.2 Antimicrobial Peptides. – In the race for supremacy over the microbes, synthetic combinatorial libraries of peptides have been generated[76] on the basis of a deconvolution strategy directed towards activity specificity. A 140-reference report[77] has appeared on nonlantiobiotic antibacterial peptides from lactic acid bacteria, and the bacteriocins from the same source have been reviewed.[78] The antibacterial peptides isolated from insects have been reviewed[79] up to 1999.

4.2.1 Antibacterial Peptides. Drosocin, pyrrhocoricin and apidaecin, representing the proline-rich antibacterials originally from insects, have been shown[80] to act in a stereospecific manner on a target bacterial protein. Biotin- and fluorescein-labelled derivatives of these peptides have assisted in the isolation of interacting proteins from *E. coli*. Drosocin is inactive *in vivo* due to rapid decomposition but pyrrhocoricin is significantly more stable[81] and analogues of this with unnatural amino acids at both termini, as well as cyclic analogues, have improved activity *in vitro*. The peptide RK-1 (**49**) a novel α-defensin related peptide has been synthesised[82] using Fmoc-solid phase protocols. S-Trt was used for protection of Cys residues and Hmb-transient protection was required to prevent aspartimide formation at Asp-Gly. An even more demanding defensin-like peptide MGD-1 (**50**) isolated from Mediterranean mussel, *Mytilus galloprovincialis*, has been synthesised[83] by solid phase. In a structural comparison with insect defensin A it is shown that they both possess a typical distribution of positively charged and hydrophobic side chains, and are the source of their antibacterial activity.



Three new peptaibols, atroviridins, A, B and C, have been identified[84] in the broth of *Trichoderma atroviride*. The structure of A has been elucidated as (**51**), with B as [Iva^{17}]-atroviridin A and C as [Aib^{6}, Iva^{17}] atroviridin A. Mixtures of microheterogeneous 16-mer peptaibol antibiotics of the antiamoebins family have been extracted[85] from the culture broths of *Stillbella* fungi. AAM (I–V) of

this family were already identified, and LC-MS techniques have provided structures for AAM (VI–XVI). The structures in the main are variations on AAM VI (**52**) having different residues at positions 1, 2, 3, 4, 5, 8 and 12. Unusually for peptaibols, the two 10-residue pseudokonins KL III (**53**) and KL VI (**54**) isolated[86] from the fungus *Trichoderma pseudokoningii* have C-termini without the usual β-amino alcohol ending. The C-terminus of (**54**) is probably the result of an intramolecular cyclisation of the two terminal residues. A β-bend ribbon structure seems to be initiated by the Xaa-Yaa-Aib-Pro motif. Trikoningins KB1 (**55**), KBII (**56**) and the L-Iva analogue (**57**) have been synthesised[87] and correlations made between conformation and membrane activity. The results confirmed the positive role played by the Iva ethyl side chain in membrane activity which was independent of its configuration. So the action on membranes is mediated through a global distribution of hydrophobic and hydrophilic residues leading to amphipathic helical structures.

Ac-Aib-Pro-Aib-Ala-Aib-Ala^{6}-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib^{17}-$(Gln)_2$-Phe-ol

(51)

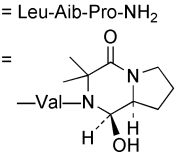
Ac-Phe-Aib-Aib-Aib-Aib-Gly-Leu-Aib-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phe-ol

(52)

Ac-Aib-Asn-Ile-Ile-Aib-Pro-Leu-R

(53) R = Leu-Aib-Pro-NH_2

(54) R =



nOct-X-Gly-Val-Aib-Gly-Gly-Val-Aib-Gly-Ile-Lol

(55) X = Aib
(56) X = D-Iva
(57) X = L-Iva

Amphibian and fish are a rich source of antibacterial peptides. Seventeen aurein peptides are present in the secretion[88] from the granular dorsal glands of Australian Bell frog *Litoria aurea* and sixteen from *L. raniformis*. Thirteen show wide-spectrum antibiotic and anti-cancer activity, and amongst the more active are aurein 1.2 (GLFDIIKKIAESF-NH_2), aurein 2.2 (GLFDIVKKVVGAL-GSL-NH_2) and aurein 3.1 (GLFDIVKKIAGHIAGSI-NH_2). Aurein 1.2 with 13 residues is the smallest aurein to show both antibiotic and anti-cancer activity (LC_{50} in 10^{-5} M range). Aurein 1.2 is shown by NMR to adopt a stable amphipathic α-helical form. ^{31}P-NMR has been used[89] to study the interaction between peptides from the skin glands of Australian tree frogs *Litoria caerulea* and *L. genimaculata*, and bacterial membranes *in vivo*. Caerin 1.1 and maculatin 1.1, both wide-spectrum antibiotics, disrupted the membranes of *B. cereus and S. epidermidis* leading to an increase in the isotropic ^{31}P signal. Infrared spectroscopy with isotopic editing has been used[90] to study interactions between the polypeptide cecropin A as it penetrated lipid membranes. The peptide adopted well-ordered secondary structure while superficially adsorbed to a membrane

surface. Novel glycosylated proteins have been isolated[91] from the epidermal mucus of tench, eel and rainbow trout. These proteins form ion-channels in lipid bilayers which correlates with a strong antibacterial activity (MIC <1 μM) for Gram positive and Gram negative bacteria.

Buforin 2 (TRSSRAGLQFPVGRVHRLLRK) from the stomach tissue of Asian toad, *Bufo gargarizans*, with a $+6$ net charge, shows antimicrobial activity[92] an order of magnitude higher than that of magainin. In the investigation of the interaction of buforin 2 with phospholipid bilayers, it was found that it exhibited much weaker membrane permeabilisation activity, and was more efficiently translocated across lipid bilayers. In a separate structure–activity analysis[93] it was discovered that the proline hinge in buforin 2 was responsible for the cell-penetrating properties. The buforins therefore constitute a new class of antibacterial peptides that target intracellular substances, most probably nucleic acids without significantly permeating cell membranes. A 20-residue hybrid peptide, KWKLFKKTGIGKFLHSAKKF-NH_2, incorporating the 1–8 residues of cecropin A and the 1–12 residues of magainin 2 has been shown[94] to have potent antimicrobial activity without toxicity against human erythrocytes. In a structure–activity study it has been revealed that the partial insertion of Trp^2 into the membrane and the positively charged Lys residues at the N-terminus leads to the primary binding to the cell membrane. Also important for activity[95] is a flexible β-turn induced by Gly-Ile-Gly or Pro creating a helix-hinge-helix structure.

Hadrurin from the venom of the Mexican scorpion, *Hadrurus aztecus* has been identified as a basic 41-amino acid peptide, GILDTIKSIASKVWNSKTVQDLKRKGINWVANKLGVSPQAA, and contains no cysteines. Its synthesis,[96] and that of two analogues, has provided the information to deduce that there appears to be no receptor molecules, and the probable mechanism is through membrane destabilisation activity. CRAMP-18 the antibacterial sequence, GEKLKKIGQKIKNFFQKL, derived from CRMAP, a member of cathelicidin-derived antimicrobial peptides, has undergone structure–activity studies.[97] By synthesising analogues designed to increase the positive charges without affecting the hydrophobic helix, it was revealed that introducing Lys at position 2 enhanced antibiotic activity. The conformation of bacteriocin AS-48, a 70-residue cyclic peptide from *Enterococcus faecalis* has been shown[98] to be a globular arrangement of five α-helices. Positive charges in a cluster within helix 4 most probably account for its antibacterial activity. A 30-residue peptide, LRVRLASHLRKLRKRLLRDADDLQKRLAVY, representing residues 133–162 of apoprotein E, has been found[99] to have antibiotic activity comparable to the classic example, gentamicin. Short synthetic sequences (15-residues) based on bovine, human, caprine, murine and porcine lactoferricins have been prepared[100] but only the bovine (MIC $=$ 24 μM) and caprine derivatives displayed measurable antibacterial activity. A number of peptide derivatives and cyclopeptides based on the N-terminal segment Arg^8-Leu^9-Val^{10}-Gly^{11} of the inhibitory centre of human cystatin C have been synthesised.[101] The cyclic analogues and derivatives of the general structure X-Arg-Leu-NH-CH(iPr)-CH_2-NH-Y had the widest spectrum of antibacterial activity. Linear (**59**) and cyclic (**58**) forms

of an amphipathic α-helical peptide, together with some diastereoisomers have been synthesised[102] and when biologically tested there was increased selectivity between bacteria and human erythrocytes in the cyclic examples. Cyclisation also abolished the oligomerisation of the linear peptide in solution and in SDS.

Peptides containing multiple dehydro amino acid and cysteine residues have been synthesised[103] in order to probe the biomimetic synthesis of the lantibiotics from their precursor peptides. Both the linear peptides (**60**) and (**61**) representing the B- and E-rings of subtilin and nisin rapidly closed stereospecifically and regiospecifically. In the case of the A-ring precursor (**62**) with two dehydro residues, regioselectivity was maintained during cyclisation, although not totally stereoselective. Some compounds contained in combinatorial libraries[104] of *N*-acetylated, *C*-amidated D-amino acid hexapeptides have been found to inhibit a bacterial 2-component signal transduction kinase.

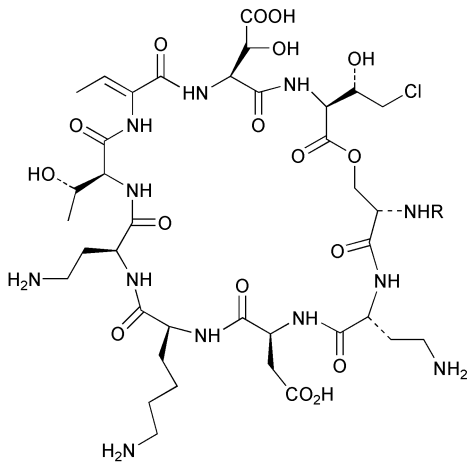
(58) (59)

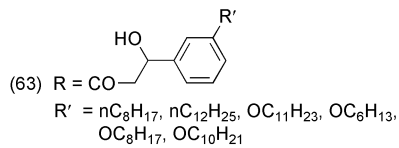
H-Leu-Dha-Pro-Gly-Cys-Val-Gly-OR
(60)
H-Leu-Dha-Ala-Asn-Cys-Lys-Ile-OH
(61)
H-Lys-Dha-Glu-Dha-Leu-Cys-Ala-OH
(62)

4.2.2 Antifungal Peptides. The potential of antifungal peptides as candidates for the treatment of fungal infections has been reviewed,[105] while the structural and biological aspects of antifungal peptides derived from chromogranins and pro-enkephalin have been discussed.[106] Synthesis and preliminary biological evaluation[107,108] of aromatic and aliphatic bearing side chain analogues of pseudomycin as exemplified by (**63**) to (**65**) showed that analogues (**63**) gave promising antifungal activity together with the n-$C_{15}H_{31}$ form of (**65**), which showed good *in vitro* activity against all three major fungi responsible for systemic fungal infections. A hexapeptide, Ac-Arg-Lys-Thr-Trp-Phe-Trp-NH_2, has been demonstrated[109] to have activity against selected phytopathogenic fungi that cause post harvest decay in fruits. Peptides synthesised were either all-D or all-L and they inhibited *in vitro* growth of *Penicillium italicum*, *P. digitatum* and *Botrytis cinera* with MICs of 60–80 μM and IC_{50} of 30–40 μM.

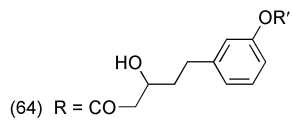
Three sets of sub-libraries[110] of an antifungal lead peptide H-His-D-Trp-D-Phe-Phe-D-Phe-Lys-NH_2 have been prepared by using variations at positions 1, 4 and 6. Potent antifungal activity against both *C. albicans* and *C. neoformans* was seen in H-Arg-D-Trp-D-Phe-Ile-D-Phe-His-NH_2. Antifungal peptides derived from the human bactericidal/permeability increasing protein (BPI) have been produced by recombinant techniques in *E. coli*.[111]

4.3 ACTH Peptides. – For some reason both manual and computer scanning of the literature under this topic have hit on just one paper during 2000. The one, takes the form of a review of the work done in the author's laboratory[112] which indicated for the first time that ACTH had a direct effect on the neuromuscular system.

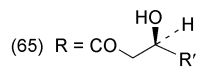




(63) R = CO
R′ = nC_8H_{17}, $nC_{12}H_{25}$, $OC_{11}H_{23}$, OC_6H_{13}, OC_8H_{17}, $OC_{10}H_{21}$



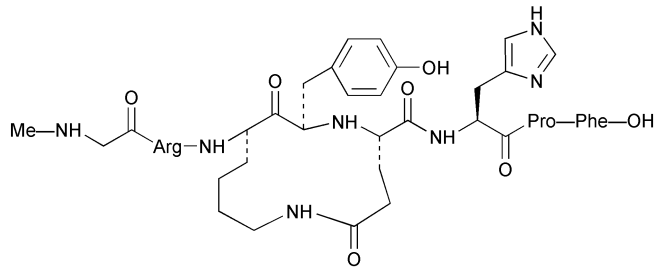
(64) R = CO
R′ = nC_5H_{11} or nC_7H_{15}



(65) R = CO
R′ = $nC_{11}H_{13}$, $nC_{15}H_{31}$ or $nC_{17}H_{35}$

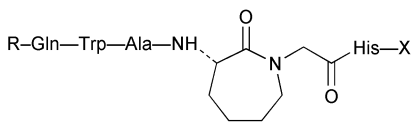
4.4 Angiotensin II Analogues and Non-peptide Angiotensin II Receptor Ligands. – The amide-linked angiotensin II potent cyclic analogue, cyclo[Sar^1,Lys^3,Glu^5]angiotensin II (**66**) has been synthesised.[113] The compound has about 15% of the activity of the parent linear peptide, probably caused by the change in the residues 3 and 5. NMR data and molecular modelling revealed a similar Tyr^4-Ile^5-His^6 bend and a His^6-Pro^7 amide in *trans* configuration to that seen in angiotensin II. Four sets of angiotensin II analogues,[114] with position 5 incorporating successively, Ile, Nle, Met, *S*-ethyl Cys, *S*-n-propyl-Cys, *S*-n-butyl-Cys and *S*-benzyl-Cys have been synthesised. Of the four, two belonged to the agonist series with either Asp or Sar in position 1 and Phe in position 8, the other two were antagonist, having Leu in position 8. On evaluating binding properties to the ATI receptor, it was deduced that the analogues bind irrespective of their agonist or antagonist nature, and that position 5 modifications without β-branching behave in an additive manner towards their affinity.

4.5 Bombesin/Neuromedin Analogues. – A glycine extended bombesin analogue[115] (*p*-hydroxy-phenylpropionyl-Gln-Trp-Ala-Val-Gly-His-Leu-Met-Gly-



(66)

OH, JMV-1458) has been tested in various biological systems. It was able to inhibit specific ^{125}I GRP binding in rat pancreatic acini and in Swiss 3T3 cells with K_i values of *ca.* 10^{-8} M. [Tyr^0, Bpa^4]-Bombesin has been synthesised[116] and on evaluation using T47D breast cancer cells it showed high affinity (K_d = 5 nM). Further binding inhibition studies confirmed that this bombesin analogue is an agonist for GRP receptors. Dipeptide and β-turn mimetics have been incorporated[117] into the bombesin sequence as illustrated in (**67–69**). Compound (**67**) showed a good affinity for GRP/BN receptors and Swiss 3T3 cells (K_i = 1.7 ± 0.4 nM and 1.0 ± 0.2 respectively). Compounds (**68**) and (**69**) with modified C-terminal residues were able to antagonise 1 nM bombesin-stimulated amylase secretion from rat pancreatic acini with high potency (K_i = 21 ± 3 and 3.3 ± 1.0 nM respectively) and 10^{-7} M bombesin-stimulated [^{3}H]thymidine incorporation into Swiss 3T3 cells (K_i = 7.8 ± 2.0 and 0.5 ± 0.1 respectively).



(67) R = H-D-Phe, X = Leu-Leu-NH_2
(68) R = *p*-hydroxyphenylpropionic acid, X = Leu-OMe
(69) R = " " " , X = NHCH-CH(OH)-$(CH_2)_3$-Me
CH_2CHMe_2

A diamine-thiol analogue of bombesin labelled with Tc-99m has been prepared.[118] Bombesin and the brain-gut axis has been discussed,[119] and in an investigation[120] of gene expression of bombesin-like peptide (BLP) and BLP receptors in lung from two baboon bronchopulmonary dysplasia (BPD) models. Both BLPs and their receptors are implicated in the pathogenesis of BPD.

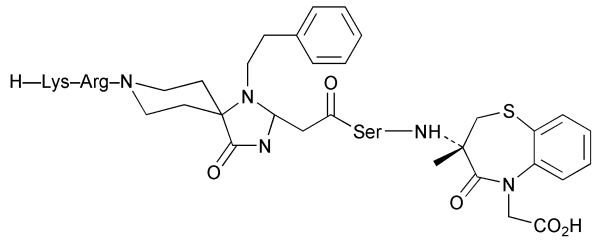
Molecular characterisation, anatomical distribution and pharmacological properties of neuromedin B have been reviewed,[121] while non-peptide antagonists have been shown[122] to interact with neuromedin B receptors inhibiting the proliferation of C6 cells. These included, 2-[3-(2,6-diisopropylphenyl)-ureido]3-(1*H*-indol-3-yl)-2-methyl-*N*-(1-pyridin-2-yl-cyclohexylmethyl)-propionate (PD 165929), 3-(1*H*-indol-3-yl)-2-methyl-2-[3(4-nitro-phenyl)-ureido]-*N*-(1-pyridin-2-yl-cyclohexylmethyl]-propionamide (PD168368) and 3-(1*H*-indol-3-yl)-*N*-[1-(5-methoxy-pyridin-2-yl)-cyclohexylmethyl]-2-methyl-2-[3-(4-nitrophenyl)-ureido]-propionamide (PD176252). All three inhibited [^{125}I-Tyr^0]-neuromedin B binding with IC_{50} values of 2000, 40 and 50 nM respectively. PD 168368 was the

best in inhibiting proliferation of C6 xenograft in nude mice *in vivo*.

The role of the two argininyl residues at positions 5 and 7 of dog neuromedin U-8 (*p*Glu-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2) has been examined[123] through the synthesis of analogues and testing them for smooth muscle contractile activity. Results revealed that Arg^7 is indispensable for receptor binding and activation to induce smooth muscle contraction. The guanidino group at position 7, but not at position 5 is strictly recognised by NMU receptors in the chicken crop. An analogue of neuromedin U, NmU-23, from the defensive skin secretion of the Australasian tree frog, *Litoria caerulea* has been shown[124] to have the structure SDEEVQVPGGVISNGYFLFRPRN-amide, and gives biological responses similar to porcine NmU-25. The North American pickerel frog, *Rana palustris*, releases[125] 22 peptides in its skin secretions, many of known structure and belonging to families such as brevinin-1, esculentin-1 and -2, ranatuerin-2 and temporin. As well as bradykinin, two peptides related to neuromedin-N (the hexapeptide KKPYIL and a larger, cystine-containing form HLRRCGKKPYILMACS) have been purified.

Neuromedin U has been shown[126] to potently activate orphan G-protein-coupled receptor FM3, and another novel G-protein-coupled receptor TGR-1, highly homologous with FM-3, has been isolated.[127] Neuromedin U specifically and clearly elevated the extracellular acidification, arachidonic acid release and intracellular Ca^{2+} mobilisation, in Chinese hamster ovary cells expressing TGR-1.

4.6 Bradykinin Analogues. – The new opportunities beckoning for bradykinin antagonists as anti-inflammatory/analgesic drugs have been highlighted.[128] In the design of B_1-receptor antagonists the dipeptide insert, D-Tic-Oic (D-BT) has proven to be a good mimic for Pro-Phe. A further analogue (JMV 1431) using this insert has now been synthesised[129] having the structure H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-BT-OH. This had affinity of 83 nM for the human-cloned B_1-receptor, but also had the ability to bind to the B_2-receptor with an affinity of 4 nM, despite the absence of a C-terminal Arg in its sequence. Retaining the insert in the C-terminal position and replacing Pro^2-Hyp^3-Gly^4-Igl^5 with the ring systems shown in (**70**) (JM1640) has given[130] an antagonist with an affinity of 24.10 ± 9.48 nM for the B_1-receptor, antagonised [des-Arg^{10}]-kallidin-induced contraction of the human umbilical vein ($pA_2 = 6.1 \pm 0.1$), but did not bind to the kinin B_2-receptor. Different interactions of peptide and non-peptide agonists and antagonists at the receptor have been put forward[131] as explanation for their pharmacology in the guinea-pig ileum. The study showed that I:I catibant (H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH), MEN 11270 (H-D-Arg-Arg-Pro-Hyp-Gly -Thi-cyclo (Dab-D-Tic-Oic-Arg) and FR 173657 [(*E*)-3-(6-acetamido-3-pyridyl)-*N*-[*N*-(2,4-dichloro-3-[(2-methyl-8-quinolyl)oxymethyl] phenyl]-*N*-methylaminocarbonylmethyl]-acrylamide gave differences in competitive *vs.* non-competitive assays which could be ascribed to their different reversibility from the B_2-receptor. Twenty previously synthesised[132] bradykinin analogues, already tested for their vasodepressor response to exogenous bradykinin, have now been tested on rat uterus. This structure–activity study appears



(70)

to support the hypothesis that there are different sub-types of B_2-receptors in rat uterus and blood vessels.

Significant differences in substrate specificity between the very similar recombinant endopeptidases EP24.15 and EP24.16 have been revealed[133] through using β-amino acid inserts into the N-terminal positions of bradykinin. β-Amino acids near the scissile Phe5-Ser^6 prevented cleavage by either enzyme, but the analogues still acted as inhibitors of the enzymes. Enhanced cleavage was evident in some analogues and not in others. Database searches using 3D-pharmacophore models have found[134] a number of new B_2-receptor antagonists, amongst libraries and commercially available compounds. N-Alkylated residues in position 2 next to Arg^1, and acylated reduced amide bonds at the penultimate residue at the C-terminus have enabled[135] backbone cyclic peptide analogues to be produced. The best results for condensation of the branching chain to the reduced peptide bond were achieved using mixed anhydrides. Linear bradykinin analogues, Lys-Lys-BK, Nle-Lys-BK and Lys-Nle-BK, together with their cyclic counterparts, and cyclo-Nle-Nle-BK and cyclo-Lys-Lys-[Trp^5]-BK have been synthesised.[136] When tested on isolated rat duodenum preparations, all except the [Trp^5]-analogue caused relaxation with EC_{50} values in the picomolar range. The most potent linear analogue, Lys-Nle-BK, was about 40 times more active than bradykinin, while the most potent cyclic counterpart, cyclo-Nle-Lys-BK, was about six times more active. Amongst the many peptides found[137] in the skin secretions of the pickerel frog, *Rana palustris*, is bradykinin itself (human version) and four peptides structurally related to [Leu^8]-BK.

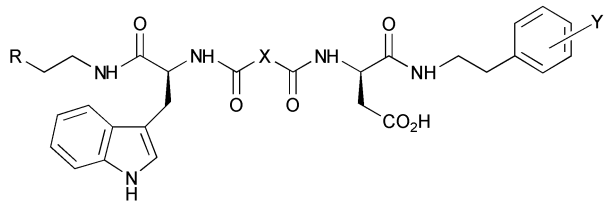
Conformational studies have been carried out on a number of bradykinin analogues. An NMR study[138] on BKM-824, cyclo(Ava-Igl-Ser-D-F5F-Oic-Arg), where Ava is 5-aminovaleric acid, Igl is α-(2-indanyl)-Gly, F5F is pentafluorophenylalanine and Oic is (2*S*,3a*S*,7a*S*)-octahydroindole-2-carboxylic acid; on BKM-g70, cyclo(D-Arg-Arg-Add-D-F5F-Oic-Arg], where Add is 12-aminododecanoic acid; and on BKM-872, cyclo(D-Arg-Arg-Eac-Ser-D-F5F-Oic-Arg),where Eac is 6-aminocaproic acid, revealed that only BKM-824 had a discernible solution structure. This molecule had a type I β-turn between residues F5F^4 and Ava^1 which differs from the usual type II′ β-turn at the C-terminal end. The importance of the N-terminal β-turn and the hydrophobic character of the C-terminus in determining the activity of bradykinin antagonists has been reinforced[139] by NMR data in 60% CD_3OH/40% D_2O on three analogues: B-10148 (Lys^{-1}-Lys^0-Arg^1-Pro^2-Hyp^3-Gly^4-Igl^5-Ser^6-D-F5F^7-Oic^8); B-10206 (D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Igl^5-Ser^6-D-F5F^7-Nc7G^8-Arg^9),where Nc7G is *N*-cycloheptyl glycine; B-10284 (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Thr^6-D-Tic^7-Oic^8-NH_2).

B-10148, a potent B_1-receptor antagonist, with high activity also on the B_2-receptor despite lacking a terminal Arg, displayed an ideal type II β-turn from Pro^2-Igl^5, and a salt bridge from the Arg^1 guanidino group to the carboxylate of Oic^8. B-10206, the most potent B_2 antagonist, also displayed the same β-turn but had little secondary structure at the C-terminus, but no definite structure could be found for B10284. In a mainly computational study[140] a β-turn in the N-terminal (2–5) is also substantiated for cyclo^{0,6}[Lys^0, Glu^6, D-Phe^7]-BK, a B_2 antagonist. A type VI β-turn was seen between Arg^1 and Gly^4 (with a *cis* Pro^2-Pro^3), a type I β-turn between Pro^2 and Phe^5 with a different type of turn covering the segment Glu^6-Arg^9.

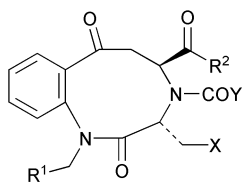
A high throughput functional assay,[141] measuring intracellular Ca^{2+} responses, showed that the most potent agonists at the B_1-receptor were des-Arg^9-BK (EC_{50} = 7.9 nM) and des-Arg^{10}-kallidin (EC_{50} = 8.6 nM), while the most potent at the B_2-receptor was bradykinin (EC_{50} = 2.0 nM). Electron spin resonance spectroscopy of several different spin labels was used[142] to study the interaction between bradykinin and analogues with anionic vesicles of dimyristoylphosphatidylglycerol. It was shown that all the peptides interact with the bilayer, making the membrane less fluid both at its surface and at the centre. Proof has been obtained[143] that the B_1-receptor is expressed in the naïve rat sensory nervous system, and the striking distribution of the receptor on sensuary neurons suggests that a direct action of B_1 activators on the nervous system may contribute to hyperalgesia.

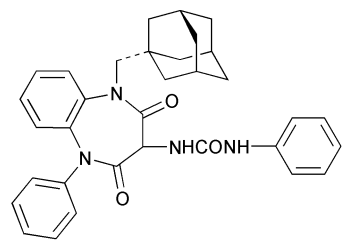
4.7 Cholecystokinin Analogues, Growth Hormone-releasing Peptide and Analogues. – In a project aimed at the management of obesity, CCK peptides combining the features of previously known hexa and tetrapeptide classes have been synthesised.[144] Hpa-Nle-Gly-Trp-Lys-Tac-Asp-MePhe-NH_2, where Hpa is 4-hydroxyphenylacetyl, has a subnanomolar affinity and 3500-fold selectivity for CCK-A receptors. It produced a long-lasting reduction in food intake in rats and a corresponding weight loss when administered over nine consecutive days. CCK-4 (Trp-Met-Asp-Phe-NH_2) has been altered[145] as a result of computer-modelling experiments. Exchanging the Met group with Pro or Nle, and modifying Phe with various substituents, led to compounds which were more effective as insulin secretagogues than CCK-4 itself. The CCK-8 analogue BC 264 (Boc-Tyr(SO_3H)-gNle-mGly-Trp-MeNle-Asp-Phe-NH_2), is known not to be anxiogenic but appears to be able to reinforce memory, but this property is not shared by CCK-4 analogues. So the C-terminal tetrapeptide of BC-264 has been developed[146] through extensions at its N-terminus *e.g.* HO_2C-CH_2-CONH-Trp-MeNle-Asp-Phe-NH_2 to yield compounds that do have BC 264 type profiles and bind to CCK_2 receptors in a specific way. The role of tyrosine sulfation and serine phosphorylation in the processing of procholecystokinin has been investigated.[147] Inhibition of tyrosine sulfation decreased the secretion of processed CCK-8, but when tyrosines were mutated to Phe, CCK was still processed and secreted. Changing Ser to Ala did not affect the processing. So there is no absolute requirement for tyrosine sulfation, although it may still be important in solubility and functional interaction.

New peptoid structures[148] based on CCK_1 tripeptide antagonists such as Boc-Trp-2-Nal-Asp-2-phenyl ethyl amide have been made, and are summarised by the general structure (**71**). Structure–activity studies carried out on this series conclude that the CCK_1 and CCK_2 receptors recognise enantiomeric dispositions of the Trp^{30} indole, the Asp^{32} COOH and C-terminal phenyl groups (within the 30–33 C-terminus of cholecystokinin) arranged about a common backbone configuration. As a consequence of molecular modelling a novel series of CCK_2 receptor antagonists, based on the diazonine template, have been made.[149] One of the best produced was compound (**72**) which was a 100-fold more selective for CCK_2 over CCK_1 receptors. A series of 5-phenyl-3-ureidobenzodazepine-2,4-diones have been synthesised[150] and evaluated as CCK-B receptor antagonists. Out of the structure–activity studies came evidence that the N-1 substituent and substitution at the urea side chain were important for CCK-B affinity, resulting in the lead compound (**73**) being taken for further development. A non-peptide CCK-1 receptor agonist PD 170292 (**74**), shows agonist activity at the high affinity sites, and as an antagonist at the low affinity sites of CCK-1 receptor.[151] It



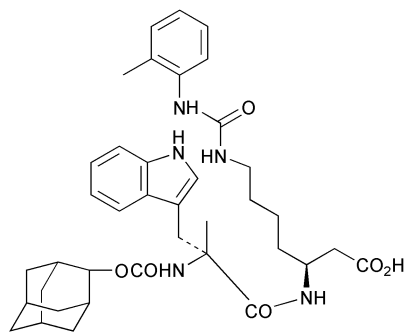
(71)





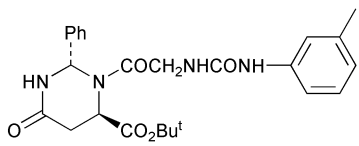
(72) R^1 = 2Cl-C_6H_4, X = l-Ad
R^2 = $NHCH_2COOH$, Y = Me

(73)

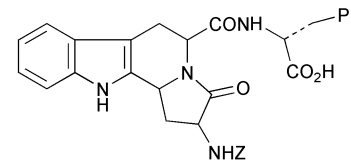


(74)

is also an efficient antagonist of CCK-2. receptor. A synthetic route has been outlined[152] for the non-peptide cholecystokinin antagonist (**75**). Replacement of the α-MeTrp residue of dipeptoids with β-turn mimetic, (2*S*,5*S*,11b*R*)-2-amino-3-oxohexahydro-indolizino[8,7-*b*]indole-5-carboxylate has led[153] to restricted analogues showing high binding affinity and selectivity for CCK-1 receptors. The analogues such as (**76**) tested in this study show nanomolar affinity for CCK-1 but are very dependent on the configuration of the β-turn mimetic insert. Using binding and affinity studies[154] based on 33-point mutated receptors it has been possible to construct molecular models for the binding of the antagonist SR 27897 and the agonist SR 146131 to the human CCK receptor. Clear differences in their binding sites have been proposed although their stuctures are very similar, SR 27897 being 1-[2-(4-(2-chlorophenyl)thiazol-2-yl)aminocarbonyl indolyl]acetic acid, and SR 146131 is 2-[4-(4-chloro-2,5-dimethoxyphenyl)-5-(2-cyclohexylethyl)-thiazol-2-ylcarbamoyl]-5,7-dimethylindol-1-yl-1-acetic acid. The synthesis and structure–activity relationships[155] of CCK-A antagonist, FK480, a CCK-B antagonist, FR 175985, and a dual CCK-A and -B antagonist, ER 208419, have been reported.



(75)



(76)

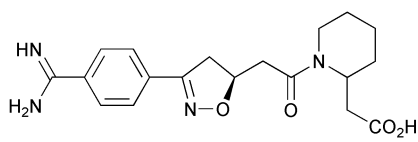
(77)

In what appears to be a lean year on productivity on growth hormone matters, the recently discovered growth hormone secretagogue, ghrelin, has been investigated.[156] Ghrelin (**77**), isolated from human gut extract, is believed to be the first example of a peptide with one of its serine residues acylated with an octanoyl group, and is a potent agonist at the human growth hormone secretagogue receptor 1a (hGHSR1a). In structure–function studies, bulky hydrophobic groups in the side chain of residue 3 turned out to be essential and short peptides encompassing the first four or five residues of ghrelin were found to activate hGHSR1a as efficiently as the full-length parent. It seems that Gly-Ser-Ser(n-octanoyl)-Phe constitutes the active core required for agonist potency at hGHSR1a.

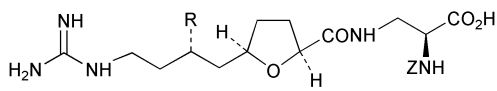
4.8 Integrin-related Peptide and Non-peptide Analogues. – This area continues to be a productive field, and a review[157] on novel parenteral and potential oral antithrombotic agents places it all in pharmaceutical context. As more and more of the compounds appear in clinical testing, a number of reviews have appeared appraising their potential as drugs. One review[158] reports pretty mixed reactions to the use of oral glycoprotein IIb/IIIa inhibition. Others[159,160] see the develop-

ments in a more positive light. An annual report[161] has appeared on anti-platelet therapies, while the orally-active platelet GPIIb/IIIa blocker, lotrafiban (SKB) has been the basis of a clinical assessment.[162]

4.8.1 IIb/IIIa Antagonists. A series of ring-constrained analogues of the GPIIb/IIIa receptor antagonist XR299 have been investigated.[163] The optimum structure found within the series, with a $IC_{50} = 0.28$ μM, was (**78**). A variety of *cis* and *trans* 2,5-disubstituted tetrahydrofuran (THF) mimetics have been prepared[164] and tested. The relative and absolute configuration of the chiral centres at the THF ring had a pronounced effect on the binding activity and selectivity. Compound (**79**) proved to be a selective inhibitor of $\alpha_{IIb}\beta_3$ ($IC_{50} = 20$ nM) whereas (**80**) exhibited high binding at $\alpha_{IIb}\beta_3$ ($IC_{50} = 67$ nM) and $\alpha_v\beta_3$ ($IC_{50} = 52$ nM). In a search[165] for low molecular weight Gp IIb/IIIa antagonists, naphthalene derivatives originating from nafamostat have shown activity. 4-(6-Amidino-2-naphthylaminocarbonyl)-phenoxyacetic acetic acid and 4-(6-amidino-2-naphthylenecarboxamido)-phenoxyacetic acetic acid inhibited adenosin-5′-diphosphate-induced aggregation of human platelet-rich plasma with IC_{50} values of 0.05 and 0.07 μM respectively. A small library of amidine-based Gp IIb/IIIa antagonists including known active compounds such as lamfiban (R044-9883) and TAK-029 have been constructed[166] *via* dendrimerised TentaGel beads using 4-amidinobenzoic acid as template. Condensed heterocyclic compounds, such as *trans*-4-(5-amidinobenzofuran-2-carboxamido)cyclohexyloxyacetic acid, and *trans*-3-[4-(5-amidinobenzo-furan-2-carboxamido)cyclohexyl]propionic acid produced[167] marked inhibitions with IC_{50} of 0.018 and 0.006 μM in a human platelet aggregation assay. However, based on its 80% inhibition in dogs, it is the ethyl *trans*-4-[5-amidinobenzofuran-2-carboxamido)-cyclohexyloxy acetate (AR05910) that has been chosen for further clinical development as an oral anti-thrombotic agent. Incorporation[168] of an Asp-Ser sequence in hirotunin to create an RGD motif, retained the anticoagulant effect of hirotunin, but had little formal disintegrin activity. Some novel anti-platelet effects were detected, however.



(78)

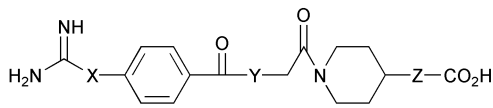


(79) 2′*S*, 5′*R* with R = H
(80) 2′*R*, 5′*R* with R = $NHCO_2Bzl$

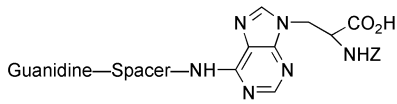
The tripeptide derivative Ac-Arg-Gly-Asp-NHMe was chosen[169] for a conformational study of the RGD sequence. Patterns and relative stabilities of H-bonds were energetically and statistically analysed using the programme

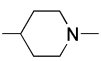
ECEPP/3 and its hydration shell model. β-Turn populations for the neutral tripeptide were reasonably consistent with NMR measurements, but conformations seem to depend on solvent polarity and pH values. Model structures such as (**81**) have been used[170] in advanced CoMFA calculations of 3D QSARs and molecular orbital calculations[171] on 2,6-amidino benzothiophenes and 5-amidinofuro[2,3-*b*]pyridines suggest the presence of preferred spatial orientation between the amidine and the carboxylic groups.

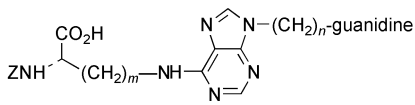
4.8.2 $\alpha_v\beta_3$ Antagonists. Purine-based peptidomimetics such as (**82**) and (**83**) have been used in libraries for the discovery of new vitronectin receptor antagonists.[172] IC_{50} values for the inhibition of kistrin binding to $\alpha_v\beta_3$ for (**82**) and (**83**) were 0.05 and 0.17 μM respectively. Hydantoin-based scaffolds such as (**84**) have assisted[173]



(81)



(82) Guanidine =  —NH—; spacer = 

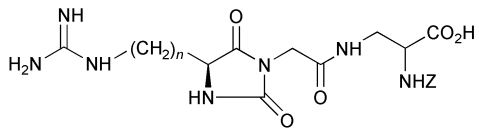


(83) Guanidine =  , $m = 1, n = 4$

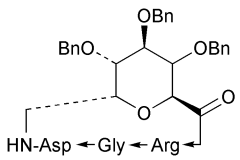
in rationalising the different binding orientations of the guanidyl groups in antagonists binding to $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$. In the former, the binding is end-on, and in the latter it is side-on. Kessler *et al.*'s highly $\alpha_v\beta_3$-selective cyclo(Arg-Gly-Asp-D-Phe-Val) has been chosen[174] as the lead structure for the development of a number of carbohydrate derivatives. As hoped for, derivatives (**85**) and (**86**) showed high $\alpha_v\beta_3$ activity (IC_{50} = 150 and 25 nM respectively). Unexpected was compound (**86**)'s activity (IC_{50} = 13.4 nM) against receptor $\alpha_{IIb}\beta_3$. This latter effect has been attributed to the greater flexibility in the conformation of the β-compound (**86**), matching the steric demands on both receptor pockets. A new series of $\alpha_v\beta_3$ integrin antagonists based on indazole have been described.[175] Indazoles attached to a 2-aminopyridine or 2-aminoimidazole by a propylene linker at the indazole 1-position and to a diaminopropionate derivative *via* a 5-carboxylate amide provided the best potency with moderate selectivity.

The key role of small molecule α_v integrin antagonists as novel anti-cancer

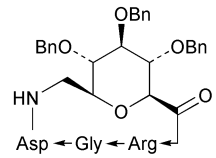
agents has been reviewed[176] and the pertinent chemistry and biological advances in the design and biological evaluation of $\alpha_v\beta_3$ antagonists as inhibitors of bone resorption has been summarised.[177]



(84) n = 1–4



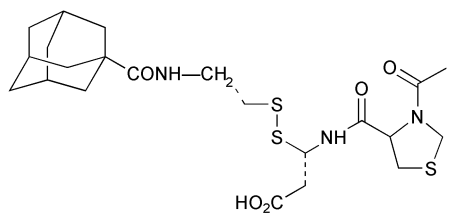
(85)



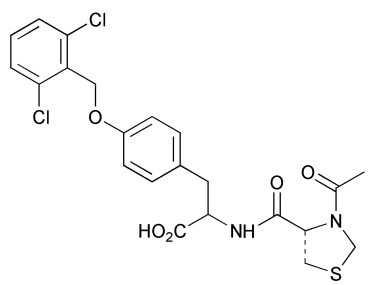
(86)

4.8.3 $\alpha_4\beta_1$ and $\alpha_5\beta_1$ Antagonists. Following on from the lead of $\alpha_4\beta_1$-antagonists based on cyclic disulfide RGD and LDV analogues, structure–activity studies[178] have revealed potent $\alpha_4\beta_1$ antagonists amongst a series of acyclic simplified structures such as (**87**). Their main disadvantage is rapid degradation ($t_{1/2}$ = 3 min). Similar potency against $\alpha_4\beta_1$ integrins has been discovered[179] amongst compounds having the structures such as (**88**). Previous proposals that fibronectin binding protein functions as a molecular bridge between M1 protein and integrin $\alpha_5\beta_1$ have been substantiated[180] through studies on the blocking of integrin $\alpha_5\beta_1$ fibronectin-M1 protein complexes by a low molecular weight non-peptide antagonist SJ755.

The $\alpha_4\beta_1$ integrin (Very Late Antigen 4,VLA-4) is expressed in many lym-

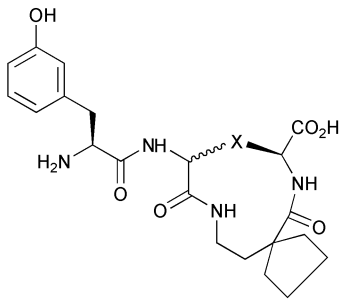


(87)

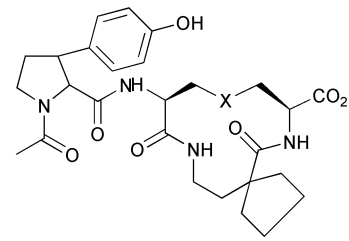


(88)

phocytes and is the principal receptor for Vascular Cell Adhesion Molecule-1, VCAM-1. Thus inhibitors of the VCAM-VLA-4 interaction could be useful for treating asthma and rheumatoid arthritis. Substitution of carbon for sulfur in a potent cyclic disulfide antagonist of the interaction has given (**89**),[181] with IC_{50} values for $n = 1$ isomers of 44 and 89 nM and values of 550 and 750 nM for $n = 2$. These are very inferior values to the parent disulfide compound. Cyclic thioether analogues such as (**90**) showed activity dependent on ring size and the position of the S atom,[182] while replacement of Asp-Pro in the original disulfide cyclic compound, with an achiral mimetic, 1-(2-aminoethyl)cyclopentyl carboxylic acid, retained[183] the IC_{50} value of 0.5 nM.



(89) X = $-CH_2-(CH_2)_n-$ n = 1 or 2



(90) X = SCH_2 or CH_2S

4.9 LHRH and GnRH Analogues. – Progress in the study of analogues of LHRH has been reviewed.[184] While agonists, functioning as anti-tumour agents for hormone-dependent tumours, have been on the market for about 10 years, antagonists are still in clinical trials. Small linear peptides, cyclic peptides and peptidomimetics have all shown *in vitro* and *in vivo* antagonist activity, with the β-turn in the central tetrapeptide and the N-terminal tripeptide segment playing an important role in receptor binding. A review[185] of the clinical development of cetrorelix, the first LHRH antagonist available clinically has been published. The rationale behind the discovery was to search for GnRH antagonists which cause inhibition of LH and FSH by competitive blockade of the receptors.

Potent hexapeptide and heptapeptide GnRH antagonists have been synthesised[186] and tested. The best heptapeptide antagonist was Ac-D-Nal2-D-Cpa-D-Pal-Gly-Arg-Pro-D-Ala-NH_2 (IC_{50} = 7 nM, *cf* GnRH, 2 nM), while the highest affinity (IC_{50} = 45 nM) for the hexapeptide Ac-D-Nal2-D-Cpa-D-Pal-Gly-Pro-D-Ala-NH_2 was lower. In order to test their effect on egg development in ovarioles (insect oostatic activity), mammalian, salmon, chicken I and II GnRHs and fragments of mammal GnRH have been synthesised[187] on 4-methylbenzhydrylamine resin using the Merrifield approach. Varying the substituents at positions 5, 7 and 8 in mGnRH (pGlu-His-Trp-Ser-Tyr^5-Gly-Leu^7-Arg^8-Pro-Gly-NH_2) showed that there was functionality associated with positions 7 and 8. Only MGnRH and two of its carboxy-truncated analogues greatly affected egg development. A series of detailed papers[188–191]have monitored the influence of constraining the GnRH structure by various cyclic bridges. A number of cyclic (4–10) and (5–8), together with bicyclic (4–10/5–8) GnRH antagonists have been

synthesised[188] and tested for affinity to the rat GnRH receptor and *in vivo* antiovulatory potency. The most potent monocyclic analogues were cyclo (4–10)[Ac-D-Nal[1], D-Fpa[2], D-Trp[3], Asp[4], D-Arg[6], Xaa[10]] GnRH with Xaa = D/L-Agl (K_i = 1.3 nM) or Dpr (K_i = 0.36 nM) which completely blocked ovulation in rats. The bicyclic analogues had only a $\frac{1}{4}$ of this potency, but varying the size of the 5–8 bridge in the bicyclic context did produce analogues with up to 200 times better affinity. It was rationalised[189] further that constriction at the N-terminus through a cyclo(1–3) scaffold would be desirable. This led to the unexpected discovery of cyclo(1–3) [Ac-D-Asp[1]. D-Cpa[2], D-Lys[3], D-Nal[6], D-Ala[10]]-GnRH (K_i = 0.82 nM), cyclo(1,1′-3) [Ac-D-Asp[1](Gly). D-Cpa[2], D-Orn[3], D-Nal[6], D-Ala[10]]-GnRH (K_i = 0.34 nM), cyclo(1,1′-3) [Ac-D-Asp[1](Gly). D-Cpa[2], D-Lys[3], D-Nal[6], D-Ala[10]]-GnRH (K_i = 0.14 nM), cyclo(1,1′-3) [Ac-D-Asp[1](β-Ala). D-Cpa[2], D-Orn[3], D-Nal[6], D-Ala[10]]-GnRH (K_i = 0.17 nM). This suggested that the N-terminal tripeptide was likely to assume a folded conformation favouring the close proximity of residues 1 and 3. The bicyclic analogue cyclo(1–3/4–10) [Ac-D-Asp[1]. D-Cpa[2], D-Lys[3], Asp[4], D-Nal[6], Dpr[10]]-GnRH had K_i = 1 nM, which suggested an unfavourable interaction between the two ring systems. NMR studies on these analogues assisted in pinpointing further avenues of constraint which would lead to a consensus model for bioactive conformations of GnRH antagonists. After many further analogues were tested[190] the best amongst them turned out to be the four bicyclic analogues, cyclo(1,1′–5/4–10)[Ac-Asp[1](Gly), D-Cpa[2], D-Trp[3], Asp[4], Dbu[5], D-Nal[6], Dpr[10]]-GnRH (K_i = 0.22 nM), cyclo(1,1′–5/4–10)[Ac-Asp[1](Gly), D-Cpa[2], D-Nal[3], Asp[4], Dbu[5], D-Nal[6], Dpr[10]]-GnRH (K_i = 0.38 nM), cyclo(1,1′–5/4–10)[Ac-Asp[1](β-Ala), D-Cpa[2], D-Trp[3], Asp[4], Dbu[5], D-Nal[6], Dpr[10]]-GnRH (K_i = 0.15 nM), and cyclo(1,1′–5/4–10)[Ac-Glu[1](Gly), D-Cpa[2], D-Trp[3], Asp[4], Dbu[5], D-Nal[6], Dpr[10]]-GnRH (K_i = 0.24 nM) However, these differed very little in structure from many others significantly less potent. So a highly discriminating receptor interaction has to be explained and after NMR studies and molecular modelling[191] a consensus bioactive conformation for cyclic GnRH antagonists has been put forward. The most prominent feature is a turn involving residues 5–8. This type II′-turn has been predicted from other theoretical studies and is allowed in the naturally occurring forms because of Gly[6] in most GnRH sequences.

4.10 α-MSH Analogues. – In a series of articles emanating from the Annals of the New York Academy of Sciences, work on α-MSH has been summarised in 'state of the art' reports. The state of available melanocortin receptor agonists and antagonists have been summarised,[192] and the mechanisms of anti-inflammatory action of α-MSH peptides have been reviewed.[193] The central and peripheral actions of α-MSH in systemic inflammation is another topic,[194] while its role in the regulation of melanocyte function has also been discussed.[195]

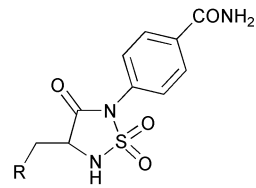
Structure–activity studies[196] of α-MSH fragments on cAMP production in incubated striatal slices revealed that the biological activity of α-MSH declines when the length of its polypeptide chain is shortened, and that the presence of Glu, as well as the core sequence, are important in any fragments' activity. The role of the aromatic and basic residues of the potent agonist (MTII) and antagon-

ist(SHU9119) at the human melanocortin receptors 3, 4 and 5 has been investigated.[197] Analogues with Glu replacing one amino acid at a time were inactive if the Glu was replacing aromatic residues. This identified the aromatic residues as the primary structural features determining interactions of the agonist/antagonist with hMCR3-5.

4.11 MHC Class I and II Analogues. – A MHC class I protein H-2K(b) has been expressed[198] on a large scale as a fusion protein with thioredoxin and hexahistidine at the N-terminus to analyse the interaction with the antigen peptide Ser-Ile-Tyr-Arg-Tyr-Tyr-Gly-Leu. NMR spectra of the peptide in the mixture solution with the protein showed very broad signals, indicating a dynamic interaction between the protein and the antigen peptide. An X-ray crystal structure[199] at 2.1 Å resolution on the ligand binding region of the inhibitory receptor LIR-1, that recognises class I MHC molecules, has shown that the binding domains are two immunoglobulin-like domains arranged at an acute angle to form a bent structure. Double-substituted peptide analogues of the tumour associated antigen MART-1(27-35) incorporating a substitution at a primary anchor residue and a β-amino acid at different positions have been synthesised.[200] In the binding with human histocompatibility class I molecule HLA-A2, the analogue [Leu^{28}, β-HIle^{30}]-MART-1(27–35) displayed both a higher affinity to HLA-A2 and a more prolonged complex stability compared to [Leu^{28}]-MART-1(27-35). So double-substitution and β-amino acid replacement at contact residues seem to give high MHC binding capacity. The crystal structure of HLA-A2 complexed with an octapeptide,Tax 8 (Leu-Phe-Gly-Tyr-Pro-Val-Tyr-Val) from human T cell lymphotropic virus-1 (HTLV-1) has been determined.[201] When compared with a higher resolution structure of HLA-A2 complexed with a nanopeptide, Tax 9, with an extra Leu at its N-terminus, the structure of the two complexes were essentially identical. In thermal stability experiments the Tax 8 complex appeared to be much less stable than its Tax 9 counterpart. A short note[202] reports the identification and immunogenicity of phosphorylated peptides associated with MHC class I molecules.

The methodology for generating a homology model[203] of the T1 T-Cell Receptor(TCR)-PBCS-K-d MHC class I complex has been presented. The construction of the model makes use of closely related homologues (the A6 TCR-Tax-HLA-A2 complex, the 2C TCR, the 14.3.d TCR V β-chain, the 1934.4 TCR V α-chain and the H-2 K-b-ovalbumin peptide), *ab initio* sampling of the complementarity determining region loop conformations and experimental data. A recently developed pairwise potential table by Betancourt and Thirumalai, describing hydrophilic interactions, has been used,[204] together with a new definition of MHC contact residues, to develop an improved algorithm for prediction of binding properties, that can be applied to a wide range of MHC class I alleles. Two new proteases in the MHC class I processing pathway have been identified,[205] and provide evidence of redundant systems acting downstream of the proteasome in the antigen-processing pathway for MHC class I molecules.

1,2,5-Thiazolidin-3-one 1,1-dioxide derivatives represented by (**91**) have been prepared[206] and their affinity for MHC class II proteins assessed. In dilute



(91) R = Ph, 2-naphthyl, $PhCH_2$ or 4-HO-C_6H_4-

solution none of the analogues had detectable affinity for HLA-DR4 proteins, but 50% inhibition was seen at more concentrated solutions (5 mM). When a C-linked isostere of β-D-galactosylated hydroxynorvaline was incorporated[207] at position 264 of the fragment CII(256-270) from type II collagen, helper T-cell hybridomas obtained in a mouse model for rheumatoid arthritis, responded when presented by class II MHC molecules. However, compared to the O-linked β-glycosylated at position 264 the C-linked isostere was 10–20 fold less active. A study[208] of peptide and peptide mimetic inhibitors of antigen presentation by HLA-DR class I MHC molecules illustrates the complementary roles played by phage display library methods, peptide analogue SAR, peptide mimetic substitution and X-ray crystallography of complexes. The active core (Leu-Arg-Met-Lys) of peptide Ii(77–92) from the immunoregulatory Ii protein has been linked[209] to an antigenic epitope of cytochrome C *via* simple spacers such as NH-$(CH_2)_4$-COO-. These hybrids were fully active and can be used to enhance vaccination with MHC class II-presented epitopes. With the availability of a 1.69 Å resolution X-ray structures[210] of superantigen staphylococcal enterotoxin H (SEH) in Zn-free and one Zn-loaded form, it has been found that the one zinc ion is bound to the C-terminal β-sheet in the region implicated for MHC class II binding. Yet the SEH/MHC class II interaction is the strongest known amongst the enterotoxins. A phagosome fraction derived from a murine macrophage cell line (J774.1), which had internalised ovalbumin (OVA)-coated latex beads, has been investigated by reversed phase HPLC.[211] Each fraction was analysed for MHC class I or II-restricted OVA-derived peptides and both were found in the phagosome fraction after less than 15 min of internalisation These results point to the vital role of phagosomes in non-cystolic antigen presentation pathway.

The capacity of the secretory pathway or of different endocytic compartments in B cell lines to generate MHC class Ii-presented peptides have been evaluated,[212] and it has been reported[213] that in naturally processed MHC class II bound peptides, residues much further away from the peptide core can modulate TCR recognition. In a study[214] on peptide-MHC class II complexes in developing dendritic cells, the transport of peptide-MHC II complexes by dendritic cells not only accomplishes transfer from late endocytic compartments to the plasma membrane, but does so in a manner that selectively concentrates TCR ligands for T-cell contact. Unexpected molecular mimicry among peptides of MHC class II, blood-clotting factor X, and HIV-1 envelope glycoprotein GP120 has been noticed.[215]

4.12 Neuropeptide Y (NPY) Analogues. – The molecular characterisation of the ligand–receptor interaction of the neuropeptide Y family has been reviewed[216] up to the end of 1999. The availability of selective compounds for the different Y-receptor sub-types are promising tools for a better understanding of the physiological properties of the NPY hormones. Another review[217] has focussed on the use of anti-receptor antibodies for the characterisation of membrane, cells and tissues, for mapping of the binding site, for purification by immunoaffinity chromatography, and for biochemical studies on G-protein-coupled receptors. An illustrative example is the characterisation of the G-protein-coupled neuropeptide Y receptor subtypes. Recent advances in the development of neuropeptide Y receptor antagonists is the subject of an annual report,[218] while an insight into the prospect of using the NPY receptor as future therapeutic targets in congestive heart failure can be gleaned from a short review.[219] The role of combinatorial chemistry in the discovery of neuropeptide Y-receptor antagonists has been noted.[220]

Structure–affinity studies have been carried out[221] on the previously known cyclooctapeptide amide cyclo[D-Cys^{29}-Cys^{34}] NPY Ac 29-36 (YM-42454) which showed high affinity for Y-1 receptors (K_i = 0.047 μM), but not for Y-2 receptors. Amino acid replacement indicated that the hydrophobic side chains Leu^{30} and Ile^{31}, the guanidinium group of Arg^{33} and Arg^{35} and the C-terminal amide are critical for the binding affinity. NMR studies indicate that these critical residues are involved in direct interaction with the Y-1 receptor rather than in maintaining the bioactive conformation. A novel restricted access cation exchanger with sulfonic acid groups at the internal surface has proven to be highly suitable in the sample clean-up of NPY peptides for LC-MS studies.[222] The chicken neuropeptide Y-2 receptor has been cloned[223] and it displays about 80% identity with the mammalian Y-2 receptor. The chicken version did not bind the mammalian selective antagonist BIIE0246 but had an unexpected high affinity for porcine[Leu^{31},Pro^{34}]-NPY. Two papers[224,225] have concentrated on the molecular evolution of the NPY family of peptides. Molecular cloning techniques to produce 3 NPY-related peptides from the sea bass (*Dicentrarchus labrax*) showed that the sequences were orthologues of both NPY, PYY and PY (fish pancreatic peptide). It has long been assumed that the first duplication to occur in vertebrate evolution generated NPY and PYY probably *via* a chromosome duplication event. Based on the work on the sea bass it seems that PY is a separate gene product, probably a duplicate of the PYY gene.

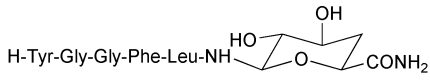
The neuropeptide Y, Y-5 receptor has been proposed as a mediator in several physiological effects of NPY, including the potent orexigenic activity of the peptide.[226] [D-Trp^{34}]-NPY turns out to be a potent and selective Y-5 receptor agonist with dramatic effects on food intake, an effect that is blocked by the selective Y-5 antagonist CGP 71683A. One theory offered to account for the co-existence of substance abuse in humans with eating disorders is that a common biochemical substrate may exist that mediates both processes. NPY peptide is believed to be one neurochemical system[227] that might contribute to these separate, yet related, problems. It has been found[227] that NPY elicits reward-related and feeding behaviour from the perifornical hypothalamus.

4.13 Opioid (Neuropeptide FF, Enkephalin, Nociceptin, Deltorphin and Dynorphin) Peptides. – Having reached the silver jubilee of their discovery, the opioids still command a great deal of interest. The developments that have led to potent, selective and stable peptide and peptidomimetic ligands have been reviewed,[228] as well as the role played by combinatorial libraries[229] in the discoveries.

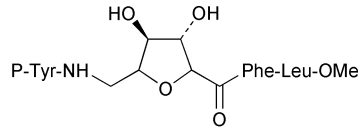
Two novel analogues[230] of neuropeptide FF have been reported. One of them (PFRF amide), increased blood pressure in a dose-dependent manner, whilst the other, [PFR(Tic)amide] decreased the blood pressure of anaesthetised rats. Research on neuropeptide FF antagonists[231] and the modulation of pain[232] has been noted. It has been hypothesised[233] that the receptor for neuropeptide FF is also a candidate for the binding of γ-2 MSH in inducing cardiovascular effects.

Topographical modifications[234] in position 1 of cyclo[D-Pen^2, D-Pen^5]-enkephalin (DPDPE) and [D-Ala^2, Asp^4]-deltorphin (DELT 1) alter the antinociceptive potency and opioid receptor selectivity. By substituting β-methyl-2,6-dimethyltyrosine in position 1, the DPDPE analogue became more susceptible to blocking by μ-antagonists, while the DELT-1 analogue was more sensitive to antagonism by δ-and μ-antagonists. While *trans*-substituted cyclopropanes are known to stabilise peptide conformations, the influence of the *cis* form has also been studied.[235] A series of *cis* cyclopropanes have been incorporated instead of Gly^2-Gly^3 and Phe^4-Leu^5 dipeptide units in Leu-enkephalin, by enantioselective cyclisation of allylic diazoacetates, catalysed by the chiral rhodium complexes Rh-2[(5*S*) MEPY]$_4$ and Rh-2[(5*R*)-MEPY]$_4$. On testing the Gly^2-Gly^3 replacement it exhibited low micromolar affinity for the μ-receptor, while the Phe^4-Leu^5 replacement did not bind to any of the opioid receptors. Twelve new [Tyr(Me)^1, Leu5]-enkephalin analogues with substituents at position 3′ of the Tyr aromatic ring have been synthesised.[236] The analogues were C-terminated with methyl esters, amides or as free acids and surprisingly in this series the esters showed higher activity to μ receptors than structurally identical C-terminal amides. The best μ-agonists in the series were [Tyr(Me)(3′-CO_2Me)^1, Leu-OMe^5]-ENK, [Tyr(Me)(3′-(E)-CH = NOH)^1, Leu-OH^5]-ENK, [Tyr(Me)(3′-(*E*)-CH = NOH)^1, Leu-NH_2^5]-ENK, [Tyr(Me)(3′-CH_2OH)^1, Leu-OMe^5]-ENK, but they were generally less active than Leu-ENK. Affinity labels suitable for δ-opioid receptors have been developed[237] by *para*-substitution of *N*,*N*-dibenzyl Leu-enkephalin. Of the target peptides tested, only *N*,*N*-dibenzyl[Phe(*p*-NCS)]-Leu enkephalin exhibited wash-resistant inhibition of radioligand binding to δ-receptors.

C-Terminal glycosyl derivatives as in (**92**) have been constructed[238] *via* solid phase techniques, with the sugar unit linked to the resin at the amide position on the sugar. The peptide was assembled on to the sugar *via* an amino group created at its 1-position. The synthesis[239] of four furanoid sugar amino acids has provided dipeptide isosteres for insertion into the Gly-Gly positions of Leu-enkephalin. The isosteres were based on 6-amino-2,5-anhydro-6-deoxy-D-gluconic acid and its mannonic, idonic and 3,4-dideoxyidonic congeners. Inserted into Leu-enkephalin they are represented by (**93**). Two of the analogues bring the aromatic rings of Tyr and Phe into close proximity, and these showed analgesic activity similar to Leu-enkephalin. A *syn* disposition of the β-hydroxycarboxyl



(92)

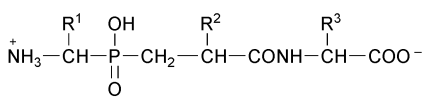


(93)

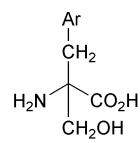
motif on the sugar rings appears to be the driving force for nucleation of the observed turn structures in the active molecules. Glycosylation provides an effective means of enhancing bioavailability and improved centrally mediated analgesia of glycosylated opioids. This approach has been applied[240] in a study of the properties of H-Tyr-cyclo[D-Cys-Gly-Phe-D-Cys]-Ser (β- or α-*O*-Glcp)-Gly-NH_2. Although binding to opioid receptors did not increase, brain uptake was improved by up to 98% for the glycosylated peptides. NMR and molecular modelling studies[241] on these glycosylated peptides showed changes in conformation at the residue of attachment (Ser^6) and on the adjacent Gly^7-NH_2. The δ-selective glycosylated Leu-enkephalin amide H-Tyr-D-Thr-Gly-Phe-Leu-Ser(β-D-Glc)-NH_2 produces[242] analgesic effects similar to morphine, yet possesses reduced dependence liability as indicated by naloxone-precipitated withdrawal studies. Conformational differences have also been found[243] on *N*-glycation of amide bonds with 6-deoxy-D-galactose at the Gly^2 position in Leu-enkephalin. FTIR spectra of the protected form of the analogue showed evidence of increased population of β-turns, an observation also observed in NMR data. An endogenous opioid peptide, MERF, (Met enkephalin-Arg^6-Phe^7) from the cerebral cortex of humans and rat has been investigated,[244] to ascertain its receptor selectivity. MERF possesses high affinity for μ-receptors, but not for δ or κ-1 and ry low affinity for κ-2 receptors in human cerebral cortex membranes. Four synthetic analogues of MERF have been prepared by solid phase synthesis[245] to achieve proteolytically more stable structures. It was concluded that MERF and its derivatives were able to activate G-proteins mainly *via* κ- and δ-opioid receptors The analogue H-Tyr-D-Ala-Gly-Phe-Met-Arg-Phe-OH turned out to be the most efficacious, equal to the parent compound.

Studies[246] on aspects of hibernation of ground squirrels has shown that the δ-opioid [D-Ala^2, D-Leu^5] enkephalin (DADLE) induces hibernation and promoted cell survival, while phosphinic derivatives such as (**94**) have been developed[247] as inhibitors of enzymes such as neprilysin and aminopeptidase N, known to inactivate enkephalins. MALDI/TOF Mass spectrometry has been used[248] to quantify the amounts of synthetic opioid, H-Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO), in ovine plasma samples and TOF/SIMS mass spectometry has monitored[249] the solid state oxidation of Met in Met-enkephalin.

A comparative molecular modelling study[250] of δ-opioid ligands has been



(94)



(95)

performed under the assumption that the potent peptide and non-peptide agonists may have a common 3D arrangement of pharmacophore groups when binding to the receptor. The δ-selective peptide used in the study was [(2*S*,3*R*)-TMT[1]]DPDPE (see ref. 234 for further details), which has a 14-membered disulfide ring and a preference for the *trans* rotamer at position 1. MD Simulations using AMBER force field and data from X-ray structures, yielded conformations similar to that seen in the crystal structure of [Ala[3]]-DPDPE, which differ significantly from the structure of DPDPE. The gauche rotamer of Phe[4] is most consistent with structure–activity of δ-opioid peptides. Other studies on the δ-opioid ligand pharmacophore have also been reviewed[251] in comparison to work done on Tyr-cyclo[D-Cys-Phe-D-Pen]-OH. The results argue against a simple view of a single common fit to a receptor binding site. The active site of Met-enkephalin has been subject to a AM1 semi-empirical quantum chemical study[252] which concludes that the Tyr phenyl group can be considered to be hydrophobic. A reference interaction site model theory[253] has been used to investigate the conformations of Met-enkephalin in methanol, ethanol and water, and X-ray data with molecular modelling have been used[254] to elucidate the conformation of the protected C-terminal dipeptide, Boc-Phe-Leu-OBzl of enkephalin.

In an attempt[255] to enhance the potency of the μ-selective tetrapeptide agonist DALDA (H-Tyr-D-Arg-Phe-Lys-NH_2), Tyr[1] has been replaced by substituted tyrosines, and Lys[4] by α,γ-diaminobutyric acid. All the resulting analogues displayed high μ-receptor selectivity with [2′,6′-dimethyl-Tyr[1]]-DALDA showing the highest potency. High affinity and selectivity for the μ-receptor was also achieved[256] through a study of residue replacement in the μ-specific tetrapeptide, Phe-cyclo(D-Cys-Phe-D-Pen)NH_2. Eleven analogues were surveyed and the preservation of the correct conformation between the aromatic rings in positions 1 and 3 was emphasised in the results. But surprisingly when Phe[1] was replaced by a cyclohexyl ring, moderate μ-affinity was still present, implying that aromaticity in that position might not be critical. All sixteen stereoisomeric analogues of endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH_2) have been synthesised.[257] Analogues containing D-residues exhibited lower interaction with μ-receptors depending on the particular combination. The consequences of introducing a hydroxymethyl entity, as in (**95**), in the form of the α-hydroxymethyl Tyr at position 1 and α-hydroxymethyl Phe at positions 3 and 4 in endomorphin-2 have been explored.[258] Significant changes in the conformations of the peptides were seen in these modifications. An analogue, H-Tyr-D-Arg-Phe-βAla-OH (TAPA) of dermorphin has been shown[259] to have a high affinity for μ-opioid receptors. From a study[260] on the μ-specific tetrapeptide Tyr-cyclo(D-Cys-Phe-D-Pen)-NH_2 (Et), where Et defines a CH_2-CH_2 within the disulfide bridge, varying Tyr[1] by replacement with conformationally restricted phenolic amino acids gave analogues with high potency at the μ-receptor (in the range 0.4–9 nM). The most selective analogue with > 1000-fold functional selectivity for the μ- over the δ-opioid receptor was D-Hat-cyclo(D-Cys-Phe-D-Pen)-NH_2 (Et), where Hat represents 6-hydroxy-2-aminotetralin-2-carboxylic acid. Based on the observation that the somatostatin analogue, octreotide, H-D-Phe cyclo(Cys-Phe-D-Trp-Lys-The-

Cys)-Thr-ol shows selectivity towards the μ-opioid receptors, small molecular mimics, based on 3-amino-3-phenylpropianamides, have been shown to exhibit high μ-affinity also.[261] The development of δ-antagonists and agonists amongst the TIPP (H-Tyr-Tic-Phe-Phe-OH) opioid peptide family has been reviewed.[262]

Interest continues in nociceptin (or orphanin FQ), (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln) the endogenous ligand for the orphan opioid-like receptor ORL-1, and in nocistatin the heptadecapeptide, Thr-Glu-Pro-Gly-Leu-Glu-Glu-Val-Gly-Glu-Ile-Glu-Gln-Lys-Gln-Leu-Gln. A three-pronged approach to structure–activity has been used[263] on nociceptin (NC), with modifications based on changes at (a) Xaa^1-Gly^2, (b) $Xaa^1\Psi[CH_2NH]Gly^2$ and (c) $Nxaa^1$-Gly^2 *i.e.* $RNHCH_2CONHCH_2CO$-NC. Modifying the steric oreientation of the aromatic ring in Phe^1 as in the series (b) leads to a reduction in efficacy, but $[Nphe^1]NC\ (1–13)NH_2$ although devoid of agonist activity behaves as a pure NC receptor antagonist, albeit weak. It is also[264] active *in vivo*, where it prevents the pronociceptive and antimorphine actions of intracerebroventricularly applied nociceptin. Both the Arg-Lys residues at positions 8–9 and 12–13 in nociceptin have been suggested to bind to an acidic amino acid cluster in the transmembrane domain of receptor ORL-1. To augment this binding a series of Arg-Lys units have been inserted[265] into positions 6–7, 10–11, or 14–15, adjacent to the parent Arg-Lys units. The former substitutions reduced activity, but $[Arg^{14}$-$Lys^{15}]$-NC was found to be very potent, and believed to be the first analogue found to be stronger than the parent nociceptin. A retro-nociceptin methyl ester, although showing weak activity for the nociceptin receptor, induces[266] analgesia and also improved learning ability. In a comparison[267] with the dynorphin A receptor, it has been worked out that in the N-terminal region of nociceptin, the distances between the aromatic residues of Phe^1 and Phe^4 are extremely critical for occupation and activation of its receptor OP4, which is in contrast to other opioid receptors. The similar order of potency of selective agonist and affinity values of a competitive antagonist for native, mouse, rat and guinea-pig receptors, suggest[268] that the same receptor is present in the four species.

A review of the binding studies performed on the nociceptin ORL receptor has been presented,[269] and in studies on the functional architecture of the receptor, it seems to be activated by interaction with the positively charged core Arg^8-Lys-Ser-Ala-Arg-Lys^{13} of nociceptin.[270] To zoom in further on the exact interaction between nociceptin and its receptor, an affinity labelling approach has been tried[271] using $[p$-benzoyl-Phe^{10}-$Tyr^{14}]$-NC. UV-Irradiation at 365 nm of the complex formed between a recombinant ORL-1 receptor and the nociceptin analogue resulted in the irreversible labelling of a glycoprotein. The photoreactive region on the receptor was identified as residues Thr^{296}-Ala-Val-Ala-Ile-Leu-Arg^{302}, with the suggestion that the p-benzoyl group was involved with the side chain of Leu.[300]

Many small molecule nociceptin mimetics have been investigated. Structure–activity studies[272] based on the known binding properties of 4-aminoquinoline derivatives with the human ORL-1 receptor picked up *N*-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethyl) benzamide hydrochloride as a

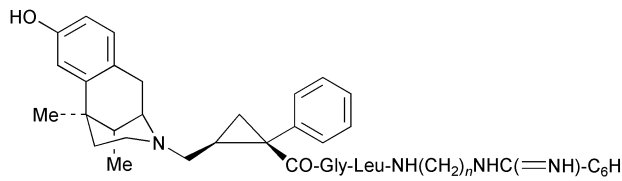
nociceptin antagonist and also showed it had an analgesic effect not antagonised by naloxone. A claim has also been made[273] that 1-[(3*R*,4*R*)-1-cycloctylmethyl-3-hydroxymethyl-4-piperidyl]3-ethyl 1,3-dihydro-2*H*-benzimidazol-2-one (J-113397) is the first potent non-peptidyl ORL1 receptor antagonist (K_1 = 1.8 nM), with high selectivity over other opioid receptors (K_1 = 1000 nM for human μ-receptor, > 10000 nM for human δ-receptor and 640 nM for the κ-opioid receptor). A 12-fold selectivity for the ORL-1 receptor over other opioid receptors has been reported[274] for the spiroxatrine analogue NNC 63-0532 (8-naphthalen-1-ylmethyl-4-oxo-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl)-acetic acid methyl ester which has a K_i = 7.3 nM. Further developments on this structural theme has found[275] (1*S*, 3a*S*)-8-(2,3,3*a*,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one to act as full agonist of the ORL-1 receptor, while even higher agonist properties with good to moderate selectivity was obtained[276] for 8-(5-methyl-1,2,3,4-tetrahydronaphthalen-1-yl)-1-phenyl-1,3,8-triazospiro[4.5]decan-4-one, together with an 8-(acenaphthen-1-yl) analogue.

Nocistatin, whose heptadecapeptide structure was given earlier, was isolated from bovine brains and cerebrospinal fluid, and its activity has been ascribed[277] to the C-terminal, Glu-Gln-Lys-Gln-Leu-Gln, which is conserved beyond species. Although nocistatin did not bind to the nociceptin receptor, it bound to the membrane of mouse brain and spinal cord with high affinity. ^{1}H NMR studies[278] on nocistatin in helicogenic solvents are consistent with a well-defined helical structure, consistent with the NMR parameters of the C-terminal octapeptide that retains allodynin-blocking activity.

A previously cited reference[234] also contains the results of placing a β-methyl-2,6-dimethyltyrosyl residue at position 1 in [D-Ala^2, Asp^4]deltorphin (DELT I). The result of this action gives an analogue more sensitive to antagonism by the δ-selective antagonist [Cys^4]deltorphin, than is the case for the parent compound. The aromatic character of Phe^3 in deltorphin II (H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2) does not seem to be critical for δ-receptor activity, as cyclohexyl and alkyl substitutions at this position give analogues which retain their activity.[279] The C-terminal Val^5-Val^6 have been recognised as having a key topographical role in δ-opioid selectivity. Replacement[280] of these residues by γ-amino acids in a QSAR study reveals a conformational stabilisation role for this domain, rather than interaction with the receptor binding pocket. Introduction[281] of Ile instead of valines at positions 5 and 6, and 6-hydroxy-2-aminotetralin-2-carboxylic acid (Hat) instead of Tyr^1 in deltorphin II gave (*R*) and (*S*)-Hat deltorphins which exhibited similar K_i values, revealing high δ-selectivity. The N-terminal deltorphin sequence (Tyr-D-Ala-Phe) has been attached[282] to polylysine and the conjugate used to immunise rabbits and a specific antibody to the tripeptide was obtained by affinity chromatography.

Dynorphin A (1–11)NH_2, H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-NH_2, since it possesses much of the activity of the parent heptadecapeptide, has been the basis for a number of structure–activity studies, especially its selectivity towards the κ-opioid receptor. Insertion[283] of Pro at positions 2 and 3 led to the discovery of analogue [Pro^3]-dynorphin A (1–11)NH_2, which although

selective for the κ-receptor (K_i ratio κ/μ/δ was 1/2110/3260), the overall receptor affinity was reduced, and it exhibits antagonistic properties. Two novel series of κ-opioid receptor agonists, analogues of MPCB-GRRI and MPCB-RRI, hybrid ligands of MPCB [(−)-*cis*-*N*(2-phenyl-2-carboxymethoxy)cyclopropylmethyl-*N*-normetazocine] and the C-terminal dynorphin A (1–8), have been synthesised.[284] Of the 40 analogues tested the two represented by (**96**) displayed high affinity and selectivity for the κ-receptor (K_i = 6.7 and 5.3 nM). A solid phase synthetic strategy[285] has been developed for the preparation of affinity labelled dynorphin analogues. Labels X = N=C=S and $NHCOCH_2Br$ were incorporated into [Phe(*p*-X)[4], D-Pro[10]]-dynorphin A(1-11)NH_2, using *p*-aminophenylalanine protected by the Alloc group, followed by group conversion on resin. Recently the historical view that Arg[6,7] and Arg[9] in the dynorphin A 'address' region interacts with acidic residues in the EL-2 receptor via ionic interaction has been questioned, and that a hydrophobic interaction is more probable. Support[286] for the latter viewpoint has come from a series of mutant forms of EL-2 in which the negative charges on anionic residues have been neutralised. These changes did not affect the affinity of dynorphin A (1–13) towards these receptors.

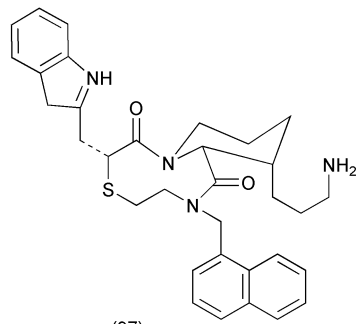


(96) *n* = 5 or 6

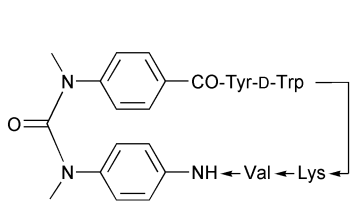
The human κ-opioid receptor interacting with the dynorphin A (1–8) sequence has undergone[287] molecular modelling studies using the bovine rhodopsin model as template together with NMR data from dynorphin A (1–14). The model generated indicates a side chain Asp[138] interaction between the receptor and the N-terminal residue of dynorphin (1–8), and returns to an ionic interaction between residues Asp[223], Glu[209] and positively charged residues in dynorphin (1–8). Molecular dynamics simulations have been applied[288] to dynorphin A(1–17) within dimyristoylphosphatidylcholine bilayers. The start-point for the simulations was evidence from NMR data derived from the peptide in dodecylphosphocholine micelles, and the overall results predict a transition in the conformation of the peptide from a parallel to a tilted orientation.

4.14 Somatostatin Analogues. – In a review[289]on the use of peptide analogues in the therapy of prostate cancer, one approach showing promise consists of cytotoxic analogues of somatostatin containing doxorubicin or 2-pyrrolinodoxorubicin. These cytotoxic analogues inhibit growth of androgen-dependent or -independent prostate cancer and reduce the incidence of metastases. A review[290] raising the question, 'can peptides be mimicked?' has relevance to this section.

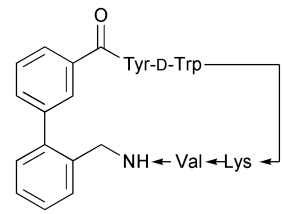
A somatostatin mimetic previously reported has undergone further structural refinement,[291] which has led to structure (**97**) exhibiting a 25-fold and 2-fold binding enhancement against somatostatin receptor sub-types, sst 4 and sst 5 respectively. Two novel β-turn mimetic models, DJS 631 (**98**), and DJS 811 (**99)** have been developed[292] to hold the bioactive somatostatin tetrapeptide in a defined conformation. Both bound selectively to somatostatin receptors sstr2b and sstr5 with affinities in the 1 nM range compared with 0.6 nM for somatostatin. Somatostatin octapeptide analogues, with backbone cyclisation *via* reduced peptide bonds as represented by (**100**) have been synthesised[293] using solid phase strategies. Stability to enzymes was improved by the bridged modification, but only moderate affinity and selectivity between receptor types was obtained. Quantitative analysis of somatostatin in pharmaceutical preparations can be made using capillary zone electrophoresis (CZE).[294]



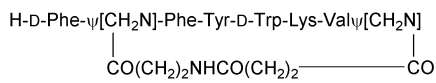
(97)



(98)



(99)

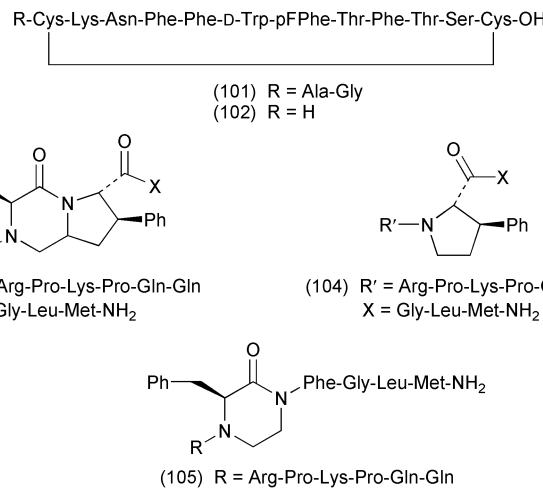


(100)

4.15 Tachykinin (Substance P and Neurokinins) Analogues. – Tachykinin-like peptides and their receptors have been reviewed,[295] while the progression of neurokinin antagonists into clinical trials have been assessed in two reports.[296,297] An update on the use of substance P(NK-1 receptor) antagonists in clinical trials on depression has appeared,[298] and the current knowledge about the function of substance P in pain has been reviewed.[299] After the failure of tachykinin NK 1 receptor antagonists to show efficacy in clinical trials of a variety of clinical pain states, questions[300] are being asked – 'why are they not analgesic in humans?'

The search to find substance P antagonists have started with a somatostatin

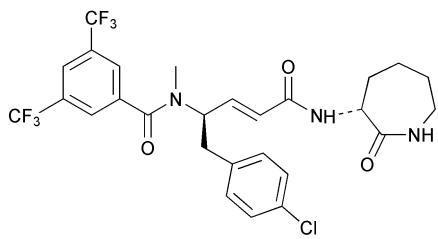
scaffold.[301] Compounds (**101**) and (**102**) were tested for their agonism/antagonism at hNK receptors and it was found that both bind to hNK_1 (220 ± 4 and 250 ± 30 nM respectively) and to hNK_2 (27 ± 4 and 46 ± 3 nM respectively). A significant synthetic effort[302] has been expended to prepare lactam-based peptidomimetic inserts for the Phe[7]-Phe[8] region of substance P as seen in (**103–105**). The building blocks proved to be completely compatible with solid phase synthesis, but analogues (**103–105**) did not show high affinity for NK-1 receptors. Since substance P represents the prototypic spinal excitatory peptide neurotransmitter/neuromodulator, acting in concert with endogenous opioid systems to regulate analgesic responses, overlapping domains from endomorphin-2 and substance P have been synthesised.[303] The chimeric sequence produced, Tyr-Pro-Phe-Phe-Gly-Leu-Met-NH_2, when administered into the rat spinal cord, produced opioid-dependent analgesia, without loss of potency over 5 days, and further assays showed the peptide could co-activate μ- and substance P receptors. This would point to a good prototype for anti-tolerance-forming analgesics with future therapeutic potential. To further elucidate the mechanism of action of neuropeptide antagonists, further studies[304] have been carried out on [D-Arg[1], D-Trp[5,7,9], Leu[11]]-substance P as an inhibitor of G-protein receptor-mediated signal transduction and cellular DNA synthesis in Swiss 3T3 cells. From the assay results it was concluded that this analogue acts as a mitogenic antagonist of the neuropeptide receptor, blocking signal transduction *via* both G(q) and G(12) sub families. In non-peptidic approaches to the search for substance P antagonists, substituted piperidines have been explored, *e.g.* the synthesis[305] of 1,1,4,4-tetra-substituted cyclopenta[*c*]piperidines and 4,4,7,7-pyrrolo[3,4-*c*] piperidines, and 4,4-disubstituted piperidine[306] *via* an oxazolidinone auxiliary resolution.



The conformational effects of adding Ca^{2+} to substance P and its [Ala[7]]-analogue within a lipid environment have been followed[307] using CD and NMR techniques. The free forms of the peptides show a helical structure in non-polar solvents, with the N-terminal region of the analogue being less ordered than its

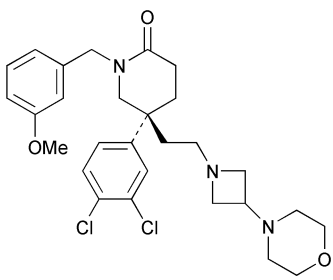
parent molecule. Addition of Ca^{2+} gave quite a significant conformational change, in both peptides, with substance P binding to two Ca^{2+} ions and Ala^7-substance P binding only to one ion. Previous studies had identified Met^{181} as the residue in the receptor NK-1 that was photo-labelled when interacted with a photoreactive substance P analogue. A similar study[308] now carried out with photo-labelled neurokinin A, together with site-directed mutagenesis of Met^{181} to Ala^{181} in the receptor, indicated that both peptides interact with the same residue on the receptor. The conformation of substance P bound to vesicles consisting of perdeuterated phosphatidylcholine has been investigated[309] by 2D transferred nuclear Overhauser spectroscopy. The N-terminal portion of the peptide seems to retain flexibility as no short or medium range NOEs were detected, but the last seven residues at the C-terminus showed non-standard turns following each other in a helix-like manner. Ranatachykinins A, B and C from bull frog brain and gut have been investigated[310] in SDS micelles using 2D-NMR techniques and molecular dynamics. Again the N-terminii showed a large degree of flexibility, but there was evidence of helicity from the mid-region to the C-terminus (4–10). A molecular dynamics simulation study[311] of substance P peptides at different membrane mimic interfaces has been carried out, and LC-MS techniques[312] have been used to analyse for the presence of the substance P antagonist ezlopitant and its metabolites in plasma.

Non-peptidic antagonists of the receptors are being intensively explored. Attempts to discover dual neurokinin NK_1/NK_2 receptor antagonists as potential anti-asthma agents have led to compound (**106**).[313] Its *in vivo* activity against NK_1/NK_2 agonist-induced bronchoconstriction in guinea-pigs gave values of $ED_{50} = 0.036$ mg kg^{-1} after 2 hr for NK_1 antagonism and 0.9 mg kg^{-1} for NK_2. The pharmacological and pharmacokinetic profile of SB-222200 [(*S*)-(−)-*N*-(α-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide], a human NK_3 receptor antagonist has been determined.[314] SB-222200 was found to be selective for the hNK_3 ($K_i = 4.4$ nM). At other receptors the values for K_i were, at $NK_1 >$ 100 000 nM and at hNK_2 250 nM. Reversibility, oral activity and CNS penetration were other valuable properties recorded for SB-222200. A key enzymatic resolution of an intermediate has enabled the synthesis of the tachykinin NK_2 antagonist (**107**) to be carried out in four steps.[315] The absolute configuration of the novel morpholine NK-receptor antagonists (**108**) and (**109**) is also critical to binding. It was the *S*,*R* forms at chiral centres * and ** which had high binding for the NK_{1-3} receptors.[316] A regio-selective method has been found[317] to phosphorylate NK_1 receptor antagonist (**110**) to produce (**111**), which can function as

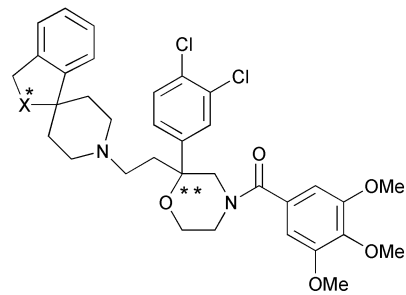


(106)

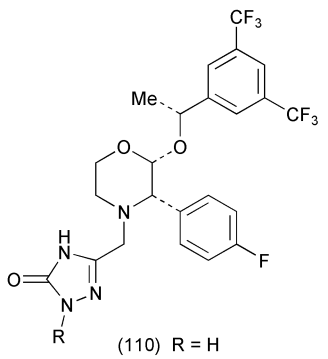
a water-soluble pro-drug of (**110**). Although the phosphorylated analogue had 10-fold lower affinity for the human NK_1 receptor, it was functionally equivalent to (**110**) *in vivo*. 4D QSAR allows for multiple-conformation orientation, protonation state ligand representation, as well as simulation of local induced-fit phenomena. Using this technique,[318] a family of 12 new receptor surrogates for the neurokinin NK_1 receptor have been validated, and four of these [congeners of (**112**)] have been synthesised. The predicted activities by calculation, matched very closely the experimental figures determined for the synthesised compounds. A new class of potent human tachykinin NK_2 receptor ligands as represented by (**114**) in Scheme 3 have been synthesised[319] *via* a stereoselctive cycloaddition between the maleic diamide (**113**) and a series of cyclic nitrones. All examples had pK_i values near to 9.0, which compare favourably with the bicyclic hexapeptide MEN 10627.



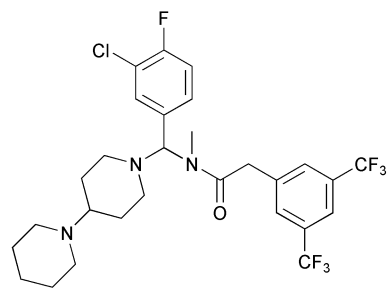
(107)



(108) X = S^+–O^-
(109) X = CHOH



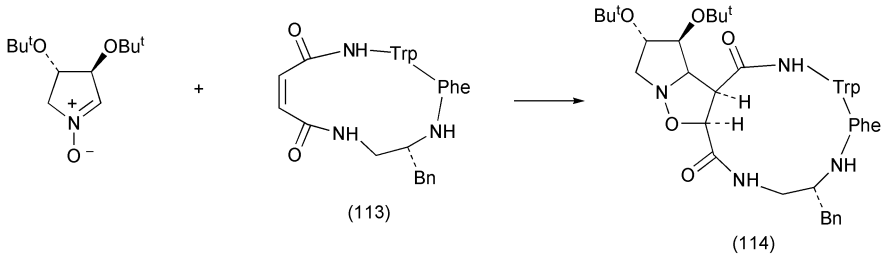
(110) R = H
(111) R = PO_3^{2-}



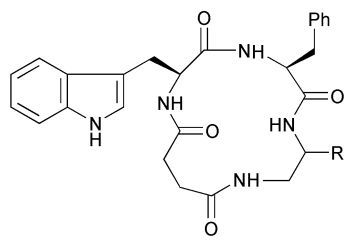
(112)

Two analogues of the dogfish-derived tachykinin, scyliorhinin (H-Ala-Lys-Phe-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2) have been investigated[320] by NMR and theoretical conformational analysis. The [MeLeu[8]]-scyliorhinin gives data to suggest a more rigid 6–10 fragment than its N-terminal region, while in the case of the analogue with a tetrazole ring between positions 7 and 8, two dominant conformers can be seen, again with very similar backbone geometry. The tetrazole ring seems to rigidify the molecule more than the *N*-methylated-

amide. The antagonistic activity profile of the cyclic pseudopeptide (**115**) against the three tachykinin receptors have convinced[321] the researchers that this should be a lead compound for further development. Site-directed mutagenesis of the NK-1 and NK-2 receptors have been used[322] to study interactions between the receptors and MDL 103 392 which is a potent dual NK-1/NK-2 antagonist. Residues important in the binding of MDL 103 392 to NK-1 were Gln^{165} His^{197}, Leu^{203}, Ile^{204} Phe^{264},His^{265} and Tyr^{272} . For binding to NK-2, Gln^{166} His^{198} Tyr^{266} and Tyr^{289} were important. All data suggest that the antagonist binds to each receptor in a different manner. The effect of substitution at the 7th position from the C-terminus of neurokinin A, on binding to the NK-2 receptor has been studied,[323] and the pharmacology of NKP608, a novel selective NK-1 receptor antagonist, has been surveyed.[324] LC-MS-MS has been used[325] to analyse for a neurokinin-1 receptor antagonist and its metabolite.



Scheme 3



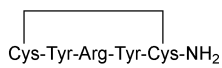
4.16 Vasopressin and Oxytocin Analogues. – *4.16.1 Oxytocin.* Analogues of oxytocin and D-homoargininyl-vasopressin have been synthesised[326] by solid phase techniques and incorporate bulky substituted Phe residues in position 2. All eight analogues with L- or D-2,3,4,5,6-pentamethyl Phe and L- or D-4-phenyl-Phe at position 2 were found to be potent inhibitors of oxytocin activity in the uterotonic *in vitro* test in the absence of Mg^{2+} ions. In order to test out the influence of *cis*-prolyl bond conformations on biological activity, 5-Bu^{t}Pro has been introduced[327] instead of Pro to form, [5-Bu^t-Pro^7]-oxytocin, [Mpa^1, 5-Bu^t-Pro^7]-oxytocin and [D-Pen^1, 5-Bu^t-Pro^7]-oxytocin. The first two analogues gave strongly reduced affinity for the receptor compared with oxytocin and also showed a higher percentage of *cis*-character in the Cys^6-Pro^7 bond. The third analogue showed stronger inhibitory potency than [D-Pen^1]-oxytocin and no

partial agonism and with some caution the authors support the hypothesis that *cis*-prolyl bond might favour antagonism while the *trans* form is necessary for agonist activity. Plasmin specifically cleaves bone matrix protein, osteocalcin, to a mid-terminal (1-44) residue peptide and a C-terminal hexapeptide, Arg-Phe-Tyr-Gly-Pro-Val, which is almost identical to the E2 region of the oxytocin receptor. So this hexapeptide was tested[328] with the osteosarcoma-associated oxytocin system, and although it was not possible to demonstrate direct binding of the hexapeptide to associated oxytocin, its presence in cultures of osteosarcoma cells antagonises the inhibitory effect of associated oxytocin on these cells.

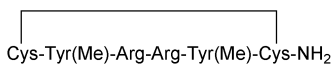
Electospray-MS has been used[329] to study the composition of coordination complexes between several transition metals and oxytocin (OT). Together with molecular modelling studies it has been proven that at pH 2 no interaction occurs, but at pH 5 the ions detected were $[OT + H^+]^+$, $[M + OT]^{2+}$, $[M + OT\text{-}H^+]^+$, $[M + OT + ClO_4^- + H^+]^{2+}$ and $[M + OT + ClO_4^-]^+$, and at pH 9 only stable 4N complexes were found. Dramatic conformational changes occur upon oxytocin coordinating to Ni-II, Mn-II or Pd-II. An artificial polymeric receptor prepared by the epitope approach of molecular imprinting has been shown[330] to recognise oxytocin in aqueous media.

4.16.2 Vasopressin. A discussion of the three receptor sub-types, V-1a, V-1b and the V-2 which mediate the biological effects of arginine vasopressin (AVP) has appeared.[331]

Six new analogues of AVP have been synthesised,[332] with D- or L-1-naphthylalanine (Nal) at positions 2 or 3. Modification resulted in a drop or the removal of antidiuretic activity, removal of pressor activity or conversion into moderate antagonists. In the latter category, [Mpa^1, L-1-Nal^2]-AVP and [Mpa^1, D-1-Nal^2]-AVP were exceptionally selective oxytocin antagonists *in vitro*. With [Mpr^1, D-Arg^8]-VP (DDAVP), already a successful drug in the treatment of diabetes insipidus and related diseases, a further stage in the research has now been reached[333] with the aim of clarifying the role of Cys^1, Tyr^2 and Arg^8 in the active centre of vasopressin. Two cyclic peptides (**116**) and (**117**), two nonapeptides representing extensions at the C-terminus with Pro-Arg-Gly-NH_2 or Pro-D-Arg-Gly-NH_2 and their Mpr^1 analogues where Mpr is β-mercaptopropionioc acid, have been prepared. All the analogues displayed low uterotonic activity *in vitro* and antidiuretic activity *in vivo*. NMR data revealed the existence of H-bonding between residues 2,5 and 3,4 in (**116**) and (**117**), but significant differences in side chain interactions within the two cyclic compounds.

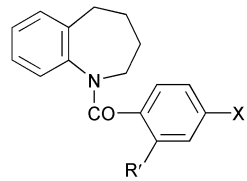


(116)



(117)

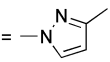
Non-peptide AVP V-2 receptor agonists, based on the benzazepine template have been syntheised[334] and tested. Amongst the most active were the analogues (**118–122**). Non-peptide human vasopressin V-2 antagonists have been produced[335] *via* the benzodiazepine scaffold in the search for molecules optimised for

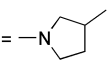


(118) R′ = Cl, X = –NH(n-Pr)

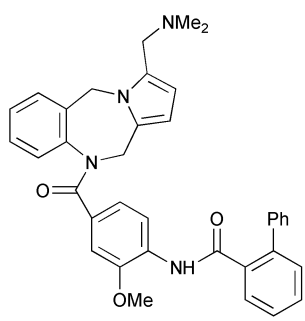
(119) R′ = H, X = 

(120) R′ = Cl, X = 

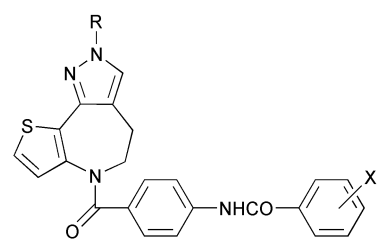
(121) R′ = Cl, X = 

(122) R′ = Cl, X = 

parenteral formulation. In this series WAY-140288 (**123**) was chosen for further development as it has an IC_{50} of 5.2 nM and a good solubility and pharmacological profile. Potent orally active AVP receptor antagonists have been based on the tricyclic heterocyclic system represented by (**124**).[336] The R group could either be H or Me but the activity was dependent on the type of substitution (X) in the benzoyl groups. Based on the hypothesis that blocking both V-1a and V-2 receptors would be useful in controlling congestive heart failure, attempts[337] have been made to produce orally active AVP antagonist for both receptors. As a result it was found that 4′-(1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzoazepine-6-carbonyl)-2-phenylbenzanilide derivatives showed potent binding affinity for both V-1a and V-2 receptors. The most potent YM087 (conivaptan) had structure, 4′-(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-*d*][1]benzoazepine-6-carbonyl)-2-phenylbenzanilide hydrochloride. In further tuning[338] of this structure for the same purpose, the analogue which exhibited exceptionally potent affinity for both receptors was (*Z*)-4′-({4,4-difluoro-5-[(4-dimethylaminopiperidino)carbonylmethylene]-2,3,4,5-1*H*-1-benzoazepin-1-yl}carbonyl)-2-phenylbenzanilide hydrochloride (YM-35471). Lipase catalysed transesterification has been instrumental[339] in enantioselectively synthesising the V-2 receptor antagonist



(123)



(124)

OPC-31260, 5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-1-benzazepine and its metabolites.

AVP and its V-1a receptor-selective antagonist d(CH_2)(5)[Tyr(Me)^2]-AVP have almost homologous sequences, differing only at residues 1 and 2, but yet have opposite effects on signal transduction. In order to determine the receptor residues responsible for discriminating the binding of agonist and antagonist ligands a site-directed mutagenesis has been carried out[340] on the transmembrane pocket of V-1a receptor. The overall deduction was that conserved aromatic residues in the binding pocket seem essential for antagonists and to not contribute to the binding of agonists. Site-specific mutagenesis has also been used[341] to rationalise why the non-peptide antagonist OPC-21268 has greater affinity for the rat V-1 receptor (V1R) than for the human V1R. Introduction of rat amino acids in positions 224, 310, 324, or 337 of the human V1R, dramatically altered OPC-21268 affinity for the receptor. This result together with computer modelling, have yielded a model for the bound ligand in which the hydrophobic part is deeply embedded in the transmembrane region, whereas the polar part is located on the surface of the extracellular side. A short sub-domain of the N-terminus of the V-1a receptor, from Glu^{37} to Asn^{47} has been recognised[342] as an absolute requirement for binding AVP and other agonists. Deleting this segment has little or no effect on binding of either peptide or non-peptide antagonists.

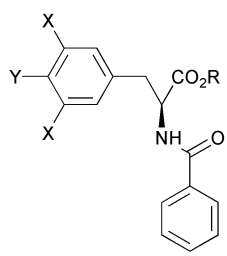
Theoretical conformational analysis using ECEPP/3 force field calculations have been carried out[343] on six AVP analogues with L-naphthylalanine at position 3. In this study on [Mpa^1,(L-1-Nal)^3,D-Arg^8]VP, [Mpa^1,(L-2-Nal)^3,D-Arg^8]VP, [(L-1-Nal)^3]AVP, [(L-2-Nal)^3]AVP, [(L-1-Nal)^3,D-Arg^8]AVP and [(L-2-Nal)^3,D-Arg^8]AVP were investigated and it was found that low energy conformations with common geometry were present in all six. In the presence and absence of water, β-turns were seen at residues Phe^3-Gln^4 and Gln^4-Asn^5, similar to the X-ray determined structures. Two-dimensional DQF-COSY and NOESY spectra of [Arg^8]-vasopressin methylenedithioether in D^6 DMSO have shown[344] that a type I β-turn in the region of Tyr^2-Asn^5 is favoured.

4.17 Insulin and Chemokines. – *4.17.1 Insulin.* The early journeys to the synthesis of insulin have been recorded,[345] while the most recent techniques, such as DNA sequencing, have proven[346] that the primary structure of fox (*Vulpes vulpes*) proinsulin is identical to dog and polar fox (*Alopex lagopus*) proinsulin. The C-terminal fragment B23-B29 of insulin, essential for the binding and function of the hormone, has undergone a detailed NMR study,[347] which revealed that the heptapeptide is monomeric in water in the concentration range 0.1–10 mM and assumes two transient conformations in addition to the *cis*/*trans* conformations about Gly^{27}-Pro^{28}. One of the transient conformations is believed to be significant in the binding of insulin to its receptor. Information about the molecular recognition event at the hormone–insulin receptor interface has been difficult to obtain. An insulin derivative labelled with the NBD fluorophore (B29 NBD-insulin) has however been used[348] to characterise the mechanism of reversible 1:1 complex formation with a fragment of the insulin receptor ectodomain. Stopped-flow fluorescence experiments showed that the kinetics of complex formation are

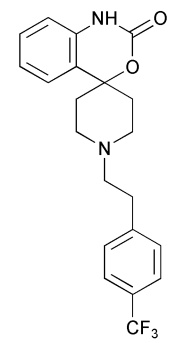
biphasic comprising a bimolecular event followed by conformational change. Two hplc methods have been developed[349] to analyse for insulin and its degaradation products in pharmaceutical preparations. Mass spectrometry[350] has been used to study site-specific hydrogen exchange properties of insulin under conditions where it is partially folded and aggregated. Slow proton exchange rates were observed in four backbone amides in the A13-19 helix region and six in the B chain helix.

4.17.2 Chemokines. This large family of chemoattractant molecules involved in the directed migration of immune cells, appear also to be involved in a variety of pro-inflammatory and auto immune diseases and they have become attractive therapeutic targets. Current efforts towards obtaining highly specific chemokine receptor antagonists have been reviewed,[351] while a short review[352] concentrates on procedures for the synthesis of chemokines.

Potent and selective non-peptidic CC chemokine receptor-3 (CCR3) antagonists have been found[353] The compounds (**125–127**), were potent inhibitors of eotaxin- and MCP-P induced Ca^{2+} mobilisation in RBL-2H3-CCR3 cells and eosinophils. A family of spiropiperidines, as represented by (**128**), have been found[354] to be effective inhibitors of chemotaxis towards MCP-1, but were poor inhibitors of CCR-1 mediated chemotaxis. It was shown that glutamate 291 in the CCR2 is a critical residue for high affinity binding, and the basic nitrogen in the spiropiperidines may be the interaction partner. The MC148 CC chemokine from human poxvirus molluscum contagiosum (MCV) has been probed[355] in parallel with viral macrophage inflammatory protein (vMIP)-II encoded by human herpesvirus 8 (HHV8) in 16 classified human chemokine receptors. HHV8 encodes the broad-spectrum chemokine antagonist vMIP-II, whereas MCV encodes a highly selective CCR8 antagonist, MC 148. Peptides mimicking chemokine receptor CCR5 have been synthesised[356] and shown to have anti-HIV-1 activities.



(125) X = I, Y = OH, R = Et
(126) X = H, Y = NO_2, R = Et
(127) X = H, Y = NO_2, R = Me

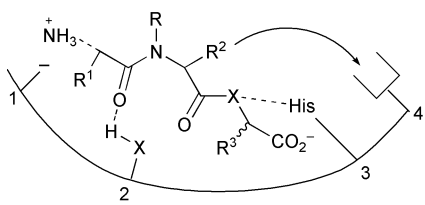


(128)

4.18 Miscellaneous. – The distribution of papers in this section this year does not readily form clusters of papers following particular themes, so sub-dividing

the section has not been possible. The search for anti-virals and nature's ability to provide molecules with specific activities were the main ambiences in many of the papers.

However, the challenge to make drugs orally active transcends all sections, so elucidating the mechanism of how the protein PepT1 transports certain drugs and small peptides is particularly interesting.[357] Several hundred PepT1 substrates have been published, and the binding and transport of about a hundred of these have been specially assessed in order to define a template for PepT1 substrates. The model interactions proposed are summarised in (**129**), which is explained as (i) strong binding for terminal NH_3^+ (ii) preference of L-configuration at R^1 (iii) planar backbone to R^2 (iv) H-bond to 1st amide bond (v) L-configuration at R^2 (vi) a hydrophobic pocket (vii) a carboxylate binding site and (viii) available space for R^3. The C-terminal pentapeptide of osteogenic growth peptide [OGP 10-14] is identical[358] to the C-terminal sequence of histone H_4 and has the sequence H-Tyr-Gly-Phe-Gly-Gly-OH, and shares OGP-like *in vitro* mitogenic effect and *in vivo* stimulation of osteogenesis and hematopoiesis. So 30 analogues have been made to study the structure–activity of the pentapeptide, with the conclusion reached that deletion of the N-terminal amino group can be tolerated, but the Tyr phenolic group, the Phe ring and the carboxyl group need to be retained for mitogenic activity. Peptide mimetics of the nerve growth factor (NGF) have been designed[359] with different types of β-turns representing the flexible C-D loop Thr[91]-Thr[92]-Asp[93]-Glu[94]-Lys[95]-Gln[96]-Ala[97]-Ala[98]. The constraint in the analogues (**130–134**) was the disulfide bridges joining the cysteine residues. Peptides which formed type I or type γL-αR β-turns were the most active. The D-retroinverso analogue (**134**) was inactive.



(129)

(130) NAcYCTDEKQ-CY

(131) NAcYCTDEKQACY

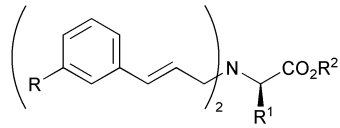
(132) NAcYC-DEKQACY

(133) NAcYC-DEKQ-CY

(134) NAcYCQKEDT-CY

Recombinant erythropoietin is the mainstay protein in the treatment of anaemia, so the challenge is on to discover a small molecule to mimic its activity. A series of *N*,*N*-dicinnamyl amino acids (**135**) have been screened[360] and have shown binding affinity with EBP ($IC_{50} < 50$ μM), but did not show erythropoietin-mimetic activity in a cell proliferation assay. A small peptide (19 amino acids) has been discovered in a random library[361] that binds to interleukin-5 receptor α/β heterodimer complex, with the same affinity to that of interleukin-5 and is a potent and specific antagonist of the interleukin in a human eosinophil adhesion assay. The active form of the peptide is a disulfide cross-linked dimer that forms spontaneously in solution.

The search for antiviral molecules continues, and the fungus KGT 142 has obliged[362] by producing two antiviral peptaibols, peptaivirins A (**136**) and B

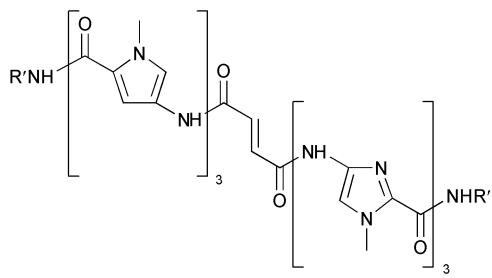


(135) R^1 = Lys, Asp or Glu

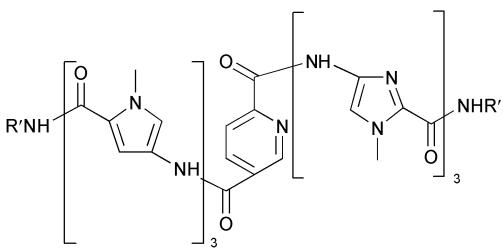
Ac-Phe-Aib-X-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Iva-Aib-Aib-Gln-Trp-ol

(136) X = Ala
(137) X = Ser

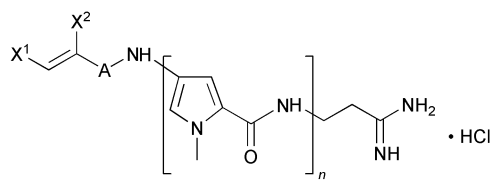
(**137**), which show strong inhibition effects of 74 and 79% at a concentration of 10 μg ml^{-1} against tobacco mosaic virus infection. The structural requirements for the strict DNA base-sequence recognition of $(AT)_4$ and $(AT)_5$ respectively for the oligopeptide minor-groove binding agent distamycin have been analysed[363] and have led to proposals for the rational structure modification for altered base recognition. This led to the synthesis of unsymmetrical imidazo-pyrrolo-bis(polyamides), related to the antiviral distamycin and bearing either unnatural termini, as in (**138**) or the natural termini as in (**139**). Halogeno-acrylic derivatives [represented by (**140**)] of distamycin A have also been synthesised[364] and have



(138) R′ = $(CH_2)_3NMe_2$

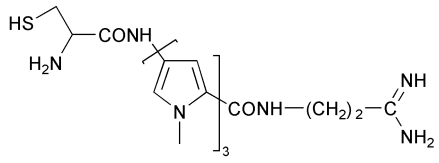


(139) R′ = $(CH_2)_2C(=NH)NH_2$

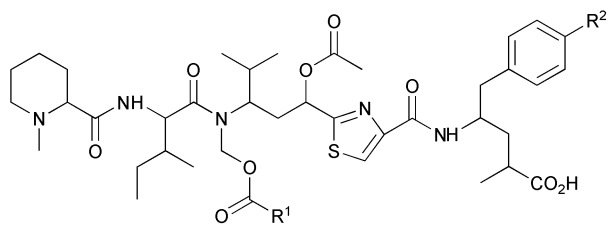


(140) X^1 = H, Cl, X^2 = Br, Cl, F, H
A = CH_2CO and *n* = 5,4,3,2 or 1

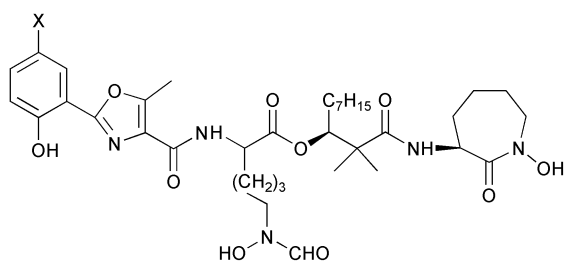
shown good cytotoxic profiles. Structures with $n = 4$ and Br attached to the acrylic group seem optimal and represent a class of minor groove binders that work in a different way from nitrogen mustards. The distamycin A/cysteine hybrid (**141**) labelled with the γ-emitting radionuclide ^{99m}Tc has been synthesised[365] as a potential radiopharmaceutical for tumour imaging in diagnostic nuclear medicine. New cytostatic peptides have been isolated[366] from the culture broth of myxobacteria *Archangium gephyra* and *Angiococcus disciformis.* Named tubulysins A, B, D and E (**142–145**), they are not active against bacteria or fungi, but showed cytostatic activity with IC_{50} in the picomolar range against mammalian cell lines. Of the two new lipopeptides, amamistatins A (**146**) and B (**147**) isolated[367] from *Nocardia asteroides* SCRS-A2359, only (**146**) inhibited growth in human tumour cell lines. Amamistatin A (**146**) has been synthesised[368] from three fragments. Synthesis of the β-hydroxy acid fragment was achieved using chiral oxazaborolidinone mediated aldol reaction, and the oxazole ring was constructed from *N*-acylthreonine *via* side chain oxidation and cyclodehydration.



(141)

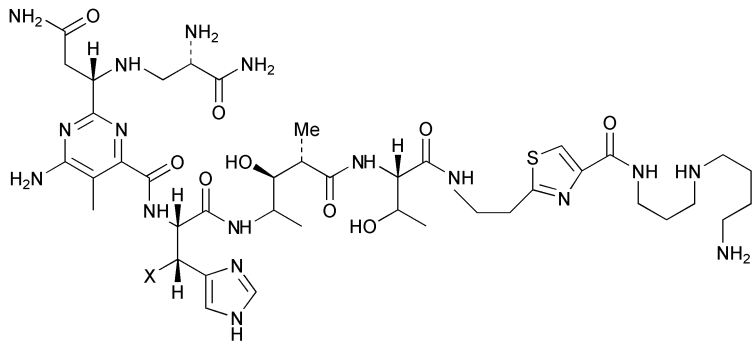


(142) $R^1 = CH_2CHMe_2$, $R^2 = OH$
(143) $R^1 = CH_2CH_2Me$, $R^2 = OH$
(144) $R^1 = CH_2CHMe_2$, $R^2 = H$
(145) $R^1 = CH_2CH_2Me$, $R^2 = H$

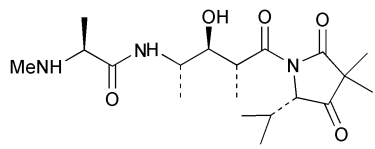


(146) X = OMe
(147) X = H

The anti-tumour antibiotics, the bleomycins, are used clinically in the treatment of several cancers. Deglycobleomycins, lacking the carbohydrate moiety of the natural product, also exhibit DNA cleavage properties similar to bleomycin itself. This has been proven by the synthesis[369] of two deglycobleomycin A_5 analogues (**148**) by solid phase peptide synthesis techniques and it was shown that the analogues relaxed supercoiled plasmid DNA to the same extent as authentic natural bleomycin A_5. A lipophilic neurotoxin, janolusimide (**149**) from the mollusc *Janolus cristatus* has been synthesised stereoselectively.[370] The mech-



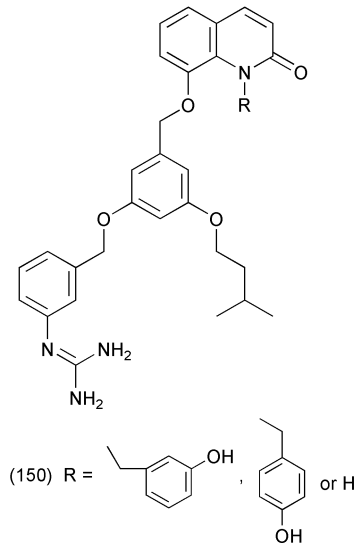
(148) X = OH or H

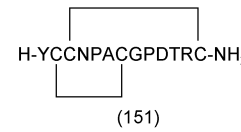


(149)

anism of action of cytotoxic peptides, functioning as small cationic molecules that modify cell membranes, either by interaction with ion-transport proteins and/or formation of ion channels has been reviewed.[371] The amphiphilic β-structure, with a polar face which interferes with microbial membranes, is a characteristic mode of action of the defensins, which have now been made available on a large scale through their large scale synthesis[372] by solid phase procedures. The mode of action of N-type calcium channel blockers, such as ω-conotoxin GVIA, is believed to involve inhibition of release of neurotransmitters. Studies[373] on rat striatum have shown that infusions of ω-conotoxin could be seen as an effective tool for the unilateral and reversible intracerebral modulation of neuronal circuits. Three mimetics (**150**) of the 25-residue peptide ω-conotoxin MVIIA have used[374] the dendroid approach to keep the key residues in conotoxin (Arg, Leu and Tyr) in a similar spatial orientation. A synthesis has been reported[375] of a new chimera peptide (**151**) formed by inserting an epitope of mucin 1 glycoprotein (MUC 1) as a guest sequence. The epitope PDTR replaces RHYS of the original conotoxin. Radio-immunoassay data showed that both linear and bicyclic forms of the chimera were recognised by MAb and HMFG1 which are both specific for the PDTR sequence.

Recent advances in the molecular biology of corticotropin releasing factor (CRF) receptors have been reviewed,[376] while a novel human calcitonin analogue has been designed[377] using computer-aided design technology. Glucagon has been systematically modified[378] by forming lactam bridges within its central region to form constrained analogues. The modifications introduced were cyclo[Asp^{9},Lys^{12}][$Lys^{17,18}$, Glu^{21}]-glucagon-NH_2, cyclo[Asp^{9},Lys^{12}]-glucagon-NH_2 , cyclo[Lys^{12}, Asp^{15}]-glucagon-NH_2, cyclo[Asp^{15},Lys^{18}]-glucagon-NH_2, Lys^{17} cyclo[Lys^{18},Glu^{21}]-glucagon-NH_2 and cyclo[Lys^{12}, Asp^{21}]-glucagon-NH_2. The first four analogues were antagonists of glucagon stimulated adenylate

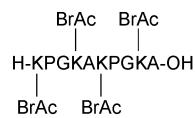




(151)

cyclase activity, whereas the two latter ones were partial agonists. All had reduced binding potencies relative to glucagon. The Template Assembled Synthetic Protein (TASP) approach has been used to create[379] analogues of the most conserved and important region of rat relaxin. Thioether linkages were used to bind peptides such as (**152**) to template T_1 (**153**), and a thiazolidine approach used to bind the peptides *via* their N-terminus. A β-sheet type conformation was demonstrated for TASP T_1(**152**)$_4$. A fragment peptide from relaxin has been a focus of study[380] into the pathways taken in metal-catalysed oxidation of His-containing peptides. Cyclo(S-S) Ac-Cys-Ala-X-Val-Gly-Cys-NH_2 was synthesised, together with its potential oxidised products where X = Asp or Asn. When studied with ascorbate/Cu(II)/O_2, none of the latter products could be detected, but the main degradation product was the cyclic peptide with X = 2-oxo-His.

(152)



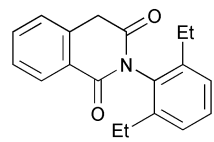
(153)

The ubiquitous 148-residue protein calmodulin is a Ca^{2+} binding protein involving a wide range of cellular Ca^{2+}-dependent signalling pathways. So in an investigation[381] designed to discover calmodulin antagonists, an all-D-hexapeptide library of peptides has been made, a typical sequence being Ac-Leu-Trp-Arg-His-Leu-Trp-NH_2. They inhibit *in vivo* cell proliferation and physical methods confirm α-helical character in their conformation. The X-ray crystallographic structure[382] of the depsipeptide, Boc-(Leu-Leu-Ala)$_2$-(Leu-Leu-Lac)$_3$-OEt confirms the presence of a 3_{10} helical segment, while the synthesis of leuprolide, the analogue of LH-RH, used for regulation of sexual hormones has been aided greatly[383] by the availability of good hplc technology. The medical and biotechnical applications of peptide nucleic acids have been reviewed.[384]

5 Enzyme Inhibitors

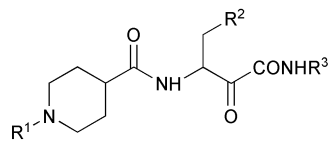
As enzyme inhibition has proved to be a fertile area of discovery for the pharmaceutical industry, it is no surprise that many areas have reached a degree of maturity, that has generated a number of reviews reflecting on the successes achieved and the prospects for the future. So a 354 reference[385] review on the current status of protease inhibitors has also within it a very useful summary table on the clinical status of many inhibitors. A good overview of the state of the art prospects for proteinase inhibitors and activators has appeared,[386] while the developments in the field of proteasome inhibitors from *in vitro* uses to clinical development has been discussed.[387] More specialised reviews on soluble neutral metallopeptidases,[388] on glycosidase inhibitors and their chemotherapeutic value,[389] and on the immunophilins (peptidylprolyl isomerases)[390] have been published.

5.1 Aminopeptidase Inhibitors. – Of the *N*-phenylphthalimide and *N*-phenylhomophthalimide derivatives assayed using human acute lymphoblastic leukemia cells, MOLT-4 with alanin-4-methylcoumaryl-7-amide as a substrate, PIQ-22 (**154**) was found[391] to be the most potent inhibitor. It also showed potent tumour-cell invasion inhibitory activity that is more effective than potent aminopeptidase inhibitors such as bestatin or actinonin. Computer-aided design of leucine aminopeptidase inhibitors has been carried out[392] using the Ligand Design (LUDI) approach to predict their activity and analyse their interactions with the enzyme. The crystal structure of bovine leucine aminopeptidase complexed with its inhibitor, the phosphonic acid analogue of leucine was taken as base structure, and from this 50 potential inhibitors, including previously discovered examples, have been highlighted.

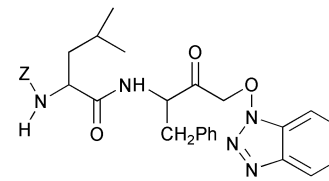


(154)

5.2 Calpain Inhibitors. – Calpain is unique among the cysteine protease family of enzymes in that it combines thiol protease activity with calmudolin-like activity. Calpain inhibitors have been grouped together in a review[393] which also discusses the impact of knowing the crystal structure of a nonpeptide calpain inhibitor bound to a hydrophobic pocket on the calcium-binding domain of calpain. Calpain inhibitors as therapeutic agents in nerve and muscle degeneration has also been discussed.[394] Piperidine carboxamides have been prepared[395] and evaluated for μ-calpain inhibition. The keto-amides (**155**) (K_i = 0.03 μM) and (**156**) (K_i = 0.009 μM) displayed a more than 100-fold selectivity over the closely related cysteine protease cathepsin B. The compounds also inhibit seizures induced by NMDA in mice. One of the highest rates of calpain inactivation has come from the inhibitor (**157**), which has a good leaving group based on N-hydroxy coupling agents. Therapeutic utility however will be restricted by their poor aqueous stability.[396] All four stereoisomers of *Z*-protected 2,3-methanoleucine have been synthesised[397] for incorporation into peptidomimetic inhibitors of calpain.



(155) R^1 = 2-naphthylCO, R^2 = Ph, R^3 = H
(156) R^1 = 2-benzothienylCO, R^2 = Ph, R^3 = H



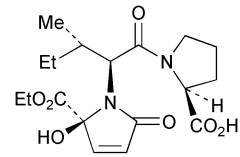
(157)

5.3 Caspase Inhibitors. – The breakdown of cellular proteins in apoptosis (programmed cell death) is mediated by the caspases, a highly conserved family of cysteine proteases. The 3D structure of the catalytic domain of caspase-9 and its interaction with the inhibitor Ac-Val-Ala-Asp-fluoromethyl ketone has now been predicted by a segment matching modelling procedure.[398] The structure shows a highly similar overall folding topology, but remarkable differences in the surface loop regions as compared to caspases-1, -3 and -8 for which crystal structures are known. Non-peptide inhibitors of caspases-3 and -7 have been identified[399] and their discovery suggests that targetting these two enzymes alone is sufficient to block apoptosis and maintain cell functionality. The isatin sulfonamide inhibitors based on an X-ray picture of their complex with caspase-3 interact with the S-2 sub-site and not the aspartic acid binding pocket (S-1), where peptide inhibitors seem to target.

5.4 Cathepsin Inhibitors. – The cathepsin family of cysteine proteases has being augmented by new arrivals. The complete nucleotide sequence of a novel cathepsin, cathepsin Q, from rat placenta has been determined.[400] The predicted structure has 343 amino acids and is related to cathepsin L. Using similar techniques the work on cathepsin K from osteoclasts has been reviewed.[401] Several novel classes of cathepsin K inhibitors have been designed based on the

X-ray co-crystal structures of peptide aldehydes bound to papain. Mouse placenta is the source[402] of cathepsin-6, whose structure bears a great deal of homology with cathepsin J, P and L. A 1.6 Angstrom resolution crystal structure of human cathepsin V, associated with an irreversible vinyl sulfone inhibitor, has been published.[403] Quite significant differences are seen in the S-2 and S-3 subsites of this enzyme compared to the active sites of related proteases. The crystal structure of human cathepsin X has been determined at 2.67 Angstom resolution.[404] Common features include a papain-like enzyme fold, but there is a pronounced feature of a mini loop that includes a 3-residue insertion protruding into the active site. Tyr^{27} on one side of the loop forms the surface of the S1 substrate binding site, and His^{23} on the other side modulates both carboxymonopeptidase as well as carboxy dipeptidase activity. The most abundant lysosomal cysteine protease, cathepsin B, becomes inactivated and undergoes denaturation at neutral or alkaline pH. NMR studies[405] have attempted to rationalise the process of inactivation and have pin-pointed a role for Cys^{29} in this deactivation process.

The 3D structure of the 56-residue Apis mellifera chymotrypsin/cathepsin G inhibitor-1 (AMCI-1) isolated from honey bee hemolymph has been calculated[406] based on 730 experimental NMR restraints. Two almost perpendicular β-sheets, several turns and a long exposed loop that includes the protease binding site, are amongst the conformational features of the peptide which has five disulfide bridges. Molecular dynamics simulations[407] of bovine cathepsin B and its complex with its specific inhibitor CA074, reveal that the existence of a Cys^{148}-Cys^{252} disulfide bond increases the flexibility of the occluding loop, although the conformational stability of the overall structure is little affected. Selectivity of CA074 stems from the tight P1′-S1′ and P2′-S2′ interactions, assisted in particular by double hydrogen bonds between the carboxyl oxygens of CA074 C-terminus and the imidazole NHs of His^{110} and His^{111} residues. The propeptide of cathepsin S inhibits cathepsin L with a K_i of 0.08 nM, yet cathepsin L propeptide inhibits cathepsin S only poorly. As a result of using chimeric propeptides, and comparing their inhibitory specificity with the wild-types, it was determined[408] that the specificity control resides in the α 1/2 helical backbone of the N-terminal parts. The propeptides of cathepsins S, L, and K have been expressed[409] as glutathione S-transferase-fusion proteins in *E. coli*, and have been tested for their inhibitory properties within the cathepsin L subfamily (cathepsins K, L and S). The cathepsin K propeptide had a K_i of 3.6–6.3 nm for each of the three cathepsins K, L and S. The cathepsin L propeptide was at least a 240-fold selective inhibitor of cathepsin K (K_i = 0.27 nM) and cathepsin L (K_i = 0.12 nM) compared with cathepsin S (K_i = 65 nM). Cathepsin S propeptide was more selective for inhibition of cathepsin L than S or K. The propeptide of cathepsin K has been shown[410] to be a potent slow inhibitor of its parent enzyme with K_i = 2.61 nM at pH 6. A set of oligoarginine peptides, derived from a combinatorial library, have turned out[411] to be specific inhibitors of cathepsin C, and the screening[412] of a combinatorial pentapeptide amide collection in an inhibition assay discovered Arg-Lys-Leu-Leu-Trp-NH_2 amongst the most potent inhibitors of cathepsin L.



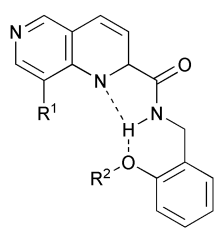
(158)

In a series of chiral 5-substituted 3-pyrrolin-2-ones synthesised[413] using a unique reaction cascade, the compound (**158**) proved to be the most potent inhibitor against cathepsin B. The same enzyme can be inhibited[414] by the peptides Pro-Phe-Pro-Gly-Pro-Ile (K_i = 2.31 mM) and Gly-Pro-Phe-Pro-Ile (K_i = 3.30 mM) isolated from a pancreatic digest of β-casein. Two affinity labels have been produced[415] for cathepsin B based on its irreversible epoxysuccinyl (Eps) inhibitor. Their structures are R-Gly-Gly-Leu-(2*S*,3*S*)-tEps-Leu-Pro-OH, with R = NH-$(CH_2)_6$-NH-rhodamine B and R = NH-$(CH_2)_6$-NH-biotin. The α-keto β-aldehyde, Z-Phe-Leu-COCHO has been shown[416] to be 400-fold more selective for cathepsin S (K_i = 0.185 nM) than for cathepsin B (76 nM), while *Z*-Phe-Tyr(OBu^t)-COCHO with a K_i of 0.6 nM is reported[417] to be the most potent synthetic reversible inhibitor of cathepsin L to date. Modified pyridoxal phosphate analogues have shown considerable inhibition of cathepsins. An analogue with the phosphate ester at position 3 replaced by propionate stongly inhibits cathepsin B, and further tuning[418] of this structure by modification of the methyl arm at position 6 has given rise to strong inhibitors of cathepsin K, weakly inhibiting cathepsin S, but with no activity on other cathepsins. A test[419] has been carried out on the effects of inhibition of cathepsin D on the production of known precursors to neurofibrillary tangles, which concluded that cathepsin D links lysosomal dysfunction to the etiology of Alzheimer's disease.

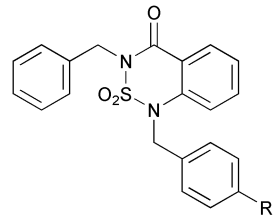
5.5 Cytomegalovirus and Rhinovirus 3C Protease Inhibitors. – A computer-based search of the literature relevant to this topic highlighted the following periodical reports as having relevance: inhibition of cysteine proteases;[420] combinatorial library synthesis 1999;[421] structure-based drug design.[422]

A new class[423] of human cytomegalovirus (HCMV) inhibitors depend on the maintenance of intramolecular H-bonds, as in (**159**), to retain activity. The range of inhibitory activity was, IC_{50} (μg mL^{-1}), 0.0004 for R^1 = Me, R^2 = i-Pr, 0.005 for R^1 = H, R^2 = i-Pr, 0.1 for R^1 = H, R^2 = Et. As part of a comprehensive study[424] of structure–activity relationships between benzothiadiazine dioxide derivatives and the inhibition of HCMV, most of the compounds, *e.g.* (**160**), exhibited anti-viral activity, some being equi-active with the standard reference ganiclovir. With further studies[425] it was found that chlorine-containing analogues were even better, so that (**160**, R = Cl) was active against a variety of HCMV clinical isolates from patients with different clinical manifestations and fully maintained its activity against a ganciclovir-resistant strain. Previous deficiences in the metabolic properties of naphthyridines and isoquinolines has spurred on research[426]on the dihydroisoquinolines such as (**161**) and (**162**). These were found to be metabolically stable and orally bioavailable.

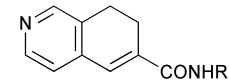
The human rhinovirus 3C protease (HRV3C) has been inactivated[427] by a



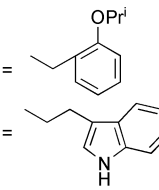
(159)



(160) R = Me, OMe, CN
CF_3 or NO_2

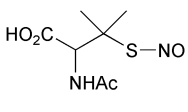


(161) R =

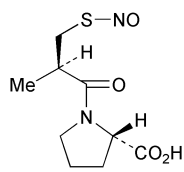


(162) R =

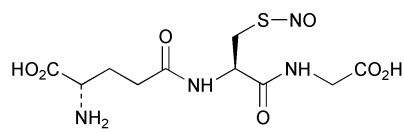
series of *S*-nitrosothiol derivatives of a number of well-known compounds, such as SNAP (**163**), *S*-nitrosocaptopril (**164**) and GSNO (**165**). The inactivation of the protease through S-nitrosylation is a transient phenomenon. Tripeptide-derived molecules incorporating C-terminal ketone electrophiles have been evaluated[428] as reversible inhibitors of HRV3C. An optimised example such as (**166**) displayed potent inhibition activity (K_i = 0.0045 μM) and *in vitro* anti-viral properties. (EC_{50} = 0.34 μM). In the design and synthesis[429] of HRV3C inhibitors, a Michael acceptor has been combined with a benzamide core, mimicking the P1 recognition element of the natural substrate. This strategy was indicated from a 1.9 Angstrom co-crystal of a benzamide inhibitor complex with the enzyme, which revealed a binding mode involving covalent attachment through the nucleophilic cysteine residue. Despite generally very modest inactivation constants being achieved, sub-micromolar activity was observed with 3-{3-carbamoyl-5-[4-(2-cyanophenyl)-piperazin-1-ylmethyl]phenyl} acrylic acid ethyl ester. The need for a Michael receptor ligand is also confirmed[430] in the inhibitors derived from bis hydrazides such as (**167**). It was only when the hydrazide was oxidised to the azo derivatives (**168**) that IC_{50}s in the low micromolar range could be achieved. A series of 3-methylthio-5-aryl-4-isothiazolecarbonitriles have been evaluated[431] as anti rhinovirus agents against a panel of 17 HRV serotypes belonging to A and B groups. Isothiazole derivatives with bulky substituents (OBn and OBut groups) in the *para*-position of the benzene ring were the most effective compounds in the series. AG7088, a novel irreversible inhibitor of HRV3C protease, was tested[432] for its antiviral activity and ability to inhibit production of IL-6 and IL-8 in a human bronchial epithelial cell line BEAS-2B. AG7088 showed a dose-dependent reduction in the levels of infectious virus and IL-6 and IL-8 released into the cell supernatant.



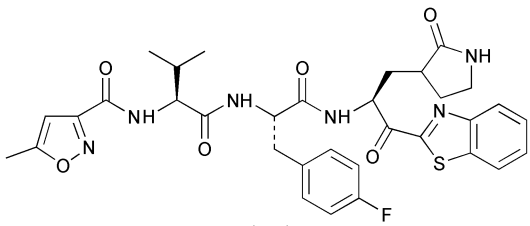
(163)



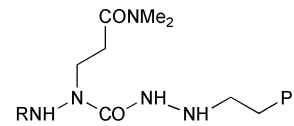
(164)



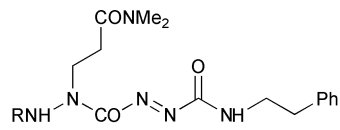
(165)



(166)



(167)

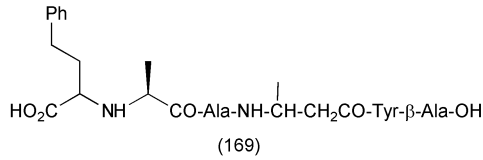


(168)

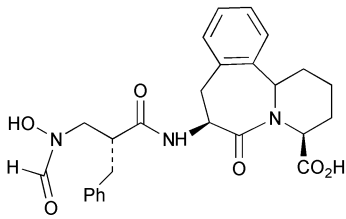
5.6 Converting Enzymes and Their Inhibitors. – *5.6.1 ACE and Related Enzymes.* The electron-conformational method of pharmacophore identification and bioactivity prediction has been applied to ACE inhibtors.[433] Prediction of the activity proved to be more than 90% within experimental error for the available compounds tried, and gave the predicted quantitative results in 60–70% of the cases. Eight inhibitors from the enalapril/lisinopril ACE inhibitor series can be separated/identified[434] by capillary electrophoresis techniques. The inhibitor CFP, *N*-[1-(*R*,*S*)carboxy-3-phenylpropyl]-Ala-Ala-Tyr-*p*-aminobenzoate is a potent and specific inhibitor of the metallo-endopeptidase EP 24.15, but is unstable due to its cleavage between Ala and Tyr by neprilysin (EP 24.11), and produces a potent ACE inhibitor as one of the degradation products, thus limiting its therapeutic use. However, incorporation[435] of a β-amino acid adjacent to the scissile bond stabilises the inhibitor against cleavage by EP 24.11. One of the best enzymically stable molecules was (**169**).

The *in vitro* and *in vivo* inhibitory potency of omapatrilat and the specific ACE inhibitor fosinopril towards the two active sites in ACE (called N- and C-domains) have been investigated.[436] *In vitro*, omapatrilat was five times more potent than fosinoprilat in inhibiting angiotensin I hydrolysis at both the N- and C-domains, but fosinoprilat was slightly more specific for the N-domain. Although compounds which inhibit the acivity of both ACE and NEP (vasopeptidase inhibitors) have been reported which incorporate a thiol, carboxylate or phosphorus acid pharmacophores, the generation of hydroxamate inhibitors has remained elusive. But that situation is no more, with the incorporation[437] of conformationally-restricted dipeptide mimetics on to a N-formyl hydroxylamine zinc-binding group to give inhibitors (**170**) and (**171**) with IC_{50} (ACE) = 3.4 and 5.3 nM and IC_{50} (NEP) = 1.8 and 1.3 nM respectively. The clinical implications of discovering a new class of drugs based on vasopeptidase inhibition has been reviewed.[438]

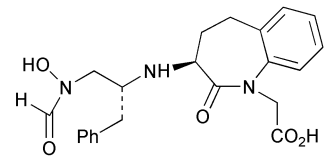
5.6.2 Endothelin Converting Enzyme. The current status and perspectives on endothelin converting enzyme (ECE) inhibitors have been reviewed.[439] ECE-1, a zinc-metalloendopeptidase generates endothelin-1 (ET-1), the most potent



(169)



(170)

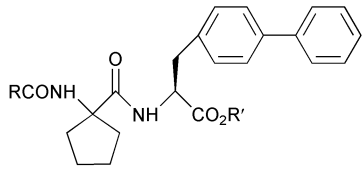


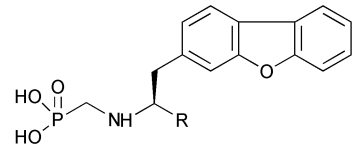
(171)

vasoconstrictor yet discovered by specific proteolytic processing of a precursor peptide big ET-1. ECE-1 cleaves big ET-1 exclusively between Trp^{21} and Val^{22}. It has now been proven[440] that in order to maintain this specificity the disulfide bonds in big ET-1 have to be in place, otherwise multi-site cleavage takes place. The minimal peptide sequence of big ET necessary for enzyme recognition and cleavage at the P1–P1′ site (Trp^{21}-Val^{22}) has been determined[441] to be big ET (18–23) amide. Asp^{18} appears to be a key residue in the recognition phenomenon. An article has appeared[442] which focuses on the 33 or so novel endothelin receptor antagonists found in patents between January 1997 and April 2000.

CGS 30084 (**172**), a known ECE-1 inhibitor at the $IC_{50} = 77$ nM level has had its structure further refined[443] to the extent that (**173**) is a long acting inhibitor of ECE-1 in rats after oral administration and with an IC_{50} of 11 nM. Sustained duration of action has also been achieved[444] with analogues of CGS 26303, the known dual ECE-1 and neutral endopeptidase (NEP) inhibitor. Replacement of its p-1′ biphenyl substituent by 3-dibenzofuranyl group has led to more potent and selective ECE-1 inhibitors such as (**174**), and the carboxylic acid (**175**), which has $IC_{50} = 22$ nM, and a 104-fold preference for ECE-1 *versus* NEP. Both the latter have been considered to have favourable activity profiles to designate them code numbers CGS 34043 and CGS 35066 respectively to carry them through further pharmacological testing. Some further results on the compounds have been published with CGS 35066 (**175**) blocking the pressor response to big ET-1 by 84%.[445] By introducing two phenyl groups at the phosphonic acid end, an orally active pro-drug was produced. CGS 34043 (**174**) has maintained its dual inhibition of ECE-1 and NEP throughout its pharmacological testing.[446] The pharmacology of SM-19712, 4-chloro-*N*-{[4-cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)amino]carbonyl}benzenesulfonamide, monosodium salt, has also looked promising,[447] with an IC_{50} value of 42 nM for ECE inhibition without having an effect at the 10–100 μM level on NEP or ACE.

5.7 Elastase Inhibitors. – The pumpkin, *Cucurbita maxima*, has been shown[448] to contain in its phloem exudate, a 42 kDa serine proteinase inhibitor having anti-elastase properties. The protein (*Cucurbita maxima* phloem serpin-1,





(172) R =  , R′ = H

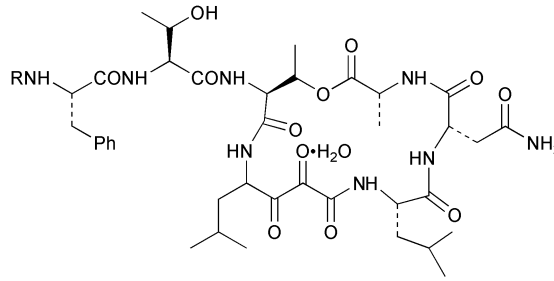
(173) R =  , R′ = Me

(174) R = 

(175) R = CO_2H

CmPS-1) has been cloned and it showed no detectable inhibitory properties against chymotrypsin, trypsin or thrombin, and the elastase cleavage sites within the reactive centre loop of CmPS-1 were determined to be Val^{347}-Gly^{348} and Val^{350}-Ser^{351}. A much simpler elastase inhibitor, SMFE102, has been isolated[449] from *Streptomyces lavendulae* SMF11 with K_i values ranging from 1.78 mM to 2.86 μM for chymotrypsin, cathepsin B and elastase. It turns out to be the dioxopiperazine, cyclo (Phe-Pro). Mass spectrometry has been shown[450] to have potential for screening for uncompetitive enzyme inhibitors, by identifying compounds which only form adducts with enzyme:substrate or enzyme:substrate analogue complexes. An example is the discovery of an octapeptide, Met-Phe-Leu-Glu-Ala-Ile-Pro-Met, from the reactive centre loop of α-1 anti-trypsin which formed a stable ternary complex with porcine elastase. The complex formed between the bicyclic peptide FR901277 from *Streptomyces resistomicificus* and porcine pancreatic elastase (PPE) has been studied[451] by X-ray crystallography and has given a structure at 1.6 Angstrom resolution. The sequence from Orn^1 to dehydroxy Thr^3 in FR 901277 formed an anti-parallel β-sheet structure with the backbone of the active site in PPE, and the S4 through S2′ binding subsites in PPE were well occupied by the hydrophobic side chains of the inhibitor molecule. FR 134043, which is a semi-synthetic disulfonated derivative of FR 910277, and an inhibitor of human leukocyte elastase, has been studied[452] by NMR spectroscopy. Confirmation was obtained of the stereochemistry being L-configuration at all C_α atoms. Total synthesis[453] has been reported for the vicinal tricarbonyl-containing elastase inhibitors YM-47141 (**176**) and YM-47142 (**177**). Key steps involved the coupling of a carboxylic acid with a phosphorane to form a stable ylide intermediate, which acts as a protecting group for the highly electrophilic carbonyl group. The CO group was 'released' by oxidative cleavage of the C=P bond. A 'one-bead, one peptide' library with 8000 variants has been constructed[454] by randomisation around the P-4, P-1 and P-2′ loop of Bowman-Birk inhibitor. Testing of the library against human leukocyte elastase, identified sequences with good inhibiton values, but surprisingly 21 out of the 23 identified sequences had Ala at P-1 and not the expected Val.

Amongst a series of pyrazolo-triazine diones tested as potential inhibitors of human leukocyte elastase, the most potent[455] turned out to be 2-*o*-methoxyphenyl-5-methyl-6-nitro-pyrazolo[2,3-*d*][1,2,4]triazine-3,7-dione,



(176) R = $COCH_2Ph$
(177) R = $CONH_2CHMe_2$

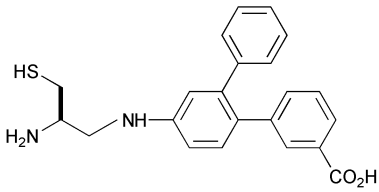
which significantly suppressed elastase-induced pulmonary injury in rats when administered orally. Acylation of the serine protease by the acyl-pyrazole is suggested as the mechanism for inhibition. Esters of clavulanic acid also acylate[456] the Ser[195] of porcine pancreatic elastase to form stable malonyl semi-aldehyde derivatives. This could develop into a general way of inhibiting serine proteases. Inhibitors of the same enzyme have been obtained[457] from other β-lactam derivatives, such as N-Boc derivatives of benzyl (*S*)-3-oxoazetidine-2-carboxylate, with K_i values in the micromolar range. Monocyclic β-lactam derivatives based on 1-carbamoyl-4- methyleneaminoxyazetidinones, in which the leaving group is the methylenaminoxy moiety, have been synthesised[458] and tested *in vitro* and *in vivo* against human leukocyte elastase. *In vitro*, some derivatives showed appreciable inhibitory activity, but *in vivo* were less promising. The synthesis and elastase inhibitory activity of phosphate esters and mixed phosphate anhydrides of penicillin sulfones have been published.[459] Amongst a series of synthesised[460] thieno[1,3]oxazin-4-ones, the most potent inhibitor of human leukocyte elastase was 2-(diethylamino)-4*H*-[1]benzothieno[2,3-*d*][1,3]oxazin-4-one with a K_i of 5.8 nM. The mechanism of inhibition is again *via* the formation of an acyl-serine intermediate. In a series of known inhibitors of chymotrypsin it has been discovered[461] that changes in the acylamino substitution increases the selectivity and potency for human leukocyte elastase, with the best inhibitory profile being reached by 6-(methylglutaryl)amino-2-[(ethylsulfonyl)oxy]-1*H*-isoindole-1,3-dione.

5.8 Farnesyltransferase Inhibitors. – Since the farnesylation of the Ras oncoprotein is required for its transforming activity in human cancer and the process is catalysed by the enzyme farnesyltransferase, the latter enzyme has become the focal point for inhibitory challenges. An update on its inhibitors has been highlighted[462] and a virtual screening[463] of a chemical database has been used to identify prototype inhibitors of farnesyltransferase. Amongst 21 compounds identified by computers, four inhibited the enzyme *in vitro* with values in the 25–100 μM range, and one inhibited the enzyme in human lung cancer cells. Farnesyltransferase inhibitors from the benzo[*f*]perhydroisoindole series have been investigated[464] using statistical design principles to identify 'activity trends' correlated with QSAR. A computer-aided conformational analysis has been carried out[465] on Cys-Val-Ile-Met, an inhibitor of farnesyltransferase. The con-

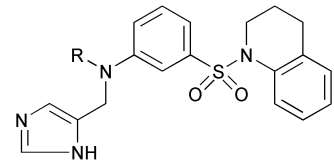
formation was computed for an aqueous environment with the peptide in the zwitterionic state. The four lowest energy conformers showed a type I bend and an extended conformation. Evaluation of the conformation of four bis-^{13}C-labelled derivatives of farnesol and geranylgeraniol derivatives reveal[466] that the prenyl side chain in a number of solvents exists primarily in an extended conformation. A MAS-solid state NMR study of the complex formed between [6,15-bis^{13}C]farnesyl diphosphate and mammalian farnesyltransferase also confirmed an extended conformation as had already been seen in X-ray-derived structures.

The ω-terminal isoprene unit in farnesyl pyrophosphate has been replaced[467] by an aniline function, to give 8-anilinogeranyl pyrophosphate, which turns out to be a substrate of farnesyltransferase, being transferred on to Ras with the same kinetics as the natural substrate. This is the first example of an alternate substrate for isoprenylation which is not an inhibitor of squalene synthase. The farnesyl pyrophosphate analogue A-176120 selectively inhibits[468] farnesyltransferase with an IC_{50} of 1.2 nM and also reduced H-ras NIH3T3 tumour growth in mice. Proliferation of cultured human smooth muscle cells has been blocked and growth factor-induced DNA synthesis has been inhibited by the farnesyl pyrophosphate analogue TR006 (IC_{50} = 67 nM).[469]

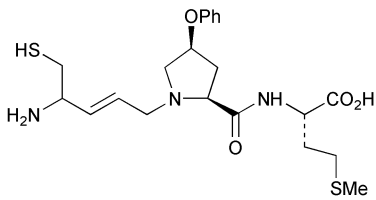
Continuing structure–activity studies[470] on imidazolyl benzodiazepine as farnesyltransferase inhibitors have unveiled the analogue BMS-214662, (*R*)-7-cyano-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1*H*-1,4-benzodiazepine as having excellent preclinical antitumour activity which has advanced it into human clinical trials. To circumvent the problems with peptide inhibitors based on the tetrapeptide CAAX the carboxy terminus of Ras protein, mimetics such as (**178**) have been synthesised[471] and tested. Compound (**178**) gave an IC_{50} of 15 nM against farnesyltransferase. An already reported piperazine-based inhibitor has been used as a template for further structural modification[472] and identified analogue (**179**), as having an IC_{50} of 0.13 μM. The conformationally constrained 8-amino-5,6,7,8-tetrahydroimidazo[1,5*a*]pyridine ring system gives improved *in vivo* metabolic stability[473] in farnesyltransferase inhibition. The mimicking of both farnesyl pyrophosphate and the CAAX tetrapeptide sequence of Ras protein into the same analogues has been accomplished[474] through the use of long-chain fatty acids and aryl-substituted carboxylic acids as farnesyl surrogates. These were then linked to Ile-NH_2, benzamide, *N*-substituted aminobenzenesulfonamides and *N*-α-aryl substitiuted methionine derivatives, which function as AAX mimetics. The amide link between the two sections of the mimetic is crucial to activity[475] as it is probably bound to the Zn ion in the active centre of the enzyme. Succinic and glutaric acid in addition to β-alanyl can function as linker groups. When benzophenone-based CAAX mimetics lose their thiol group, *e.g.* by a substitution with a COOH group, inhibition of farnesyltransferase diminishes,[476] but by adding a lipophilic alkyl chain to the molecule it regains inhibitory activity. CAAX Mimetics based on HOPro as in (**180**) have been assessed[477] and give low micromolar IC_{50} values in inhibition of farnesylation. The recently discovered farnesyltransferase inhibitors kurasoins A and B have been synthesised[478] asym-



(178)



(179) R = 4-(5)-imidazolymethyl

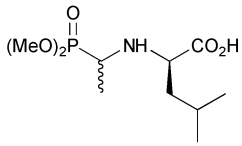


(180)

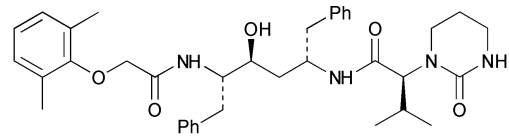
metrically in seven steps from 2-(4-hydroxyphenyl)ethanol and have shown that the stereochemistry of the hydroxy group is important for inhibitory activity. The pharmokinetics–antitumour activity relationships have been worked out[479] for the novel inhibitor BMS-214662.

5.9 HIV Protease Inhibitors. – HIV-Protease inhibitors have been discussed[480] and a computer analysis[481] of superimposed crystal structures for 266 small molecules bound to 48 proteases from the four main categories (aspartic, serine, cysteine and metallo) has given the first proof that inhibitors commonly bind in an extended β-strand conformation at the active sites of all proteases. This conclusion was supported by NMR, CD and binding studies for HIV-1 protease inhibitors/substrates, which when pre-organised in an extended conformation have significantly higher protease affinity.

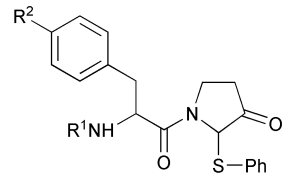
With the synthesis[482] of α-aminophosphonic acid derivatives, formation of (**181**) has been made possible, which is now undergoing assessment as HIV-protease inhibitors. A large scale synthesis[483] has been published for the HIV-protease inhibitor ABT-378 (lopinavir, **182**). Some pretty potent inhibitors such as (**183–185**) have been obtained[484] with IC_{50} (μM) values of 0.008, 0.02 and 0.026 respectively, by replacing Pro with 2-thiophenoxy-3-pyrrolidine in Phe-Pro dipeptides. The influence of the central hydroxyl groups on the anti-viral activity of the symmetrical HIV-1 protease inhibitor (**186**) has been examined.[485] Deletion of any one of the hydroxyls diminishes the inhibitor activity and for optimal inhibition the analogue with hydroxyls in the C-3*R* and C-4*R* configurations was best. A new and highly potent cyclic HIV protease inhibitor scaffold has been discovered[486] through synthesis. The best analogue (**187**) in the series was roughly equipotent with marketed HIV-protease inhibitors at that time. A unique and novel amino acid, allo-phenylnorstatine, with a hydroxymethylcarbonyl isostere as the active moiety has been incorporated[487] into a tripeptide-based orally potent inhibitor. Analogue (**188**), KNI-272, had a good enough profile (K_i = 6.6 nM) to undergo clinical trials, and two long-lasting analogues of this compound, (**189**) and (**190**) were also found.



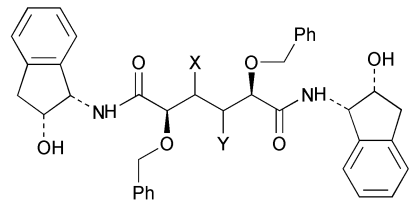
(181)



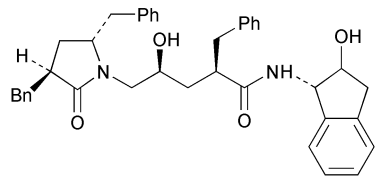
(182)



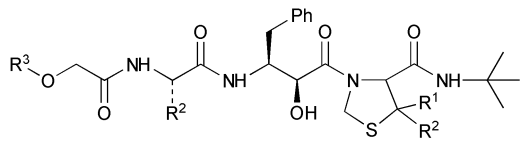
(183) R^1 = Boc, R^2 = H
(184) R^1 = Boc, R^2 = OBn
(185) R^1 = Boc, R^2 = OH



(186)

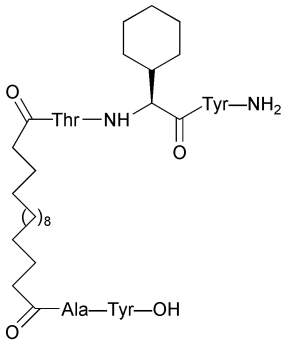


(187)

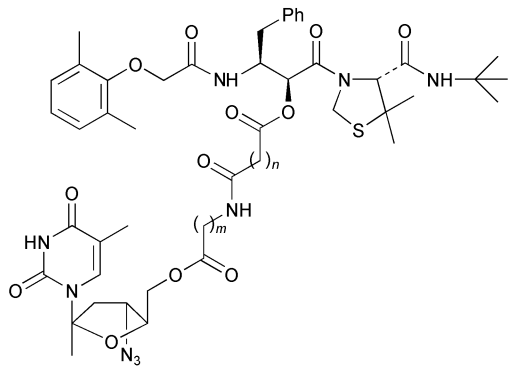


(188) R^1 = H, R^2 = CH_2SMe, R^3 = 5-isoquinolyl
(189) R^1 = Me, R^2 = i-Pr, R^3 = 3-dimethyl-aminophenyl
(190) R^1 = Me, R^2 = Et, R^3 = 3-dimethyl-aminophenyl

In a new approach, involving targetting the dimerisation interface of HIV-protease, the minimum sequence of amino acids needed to effect inhibition was worked out[488] and the inhibitor (**191**) (IC_{50} = 680 nM), was very near to the smallest structural requirement. A 'double drug' strategy has been used[489] to enhance anti-viral activity and involves combining a HIV-protease inhibitor and a nucleoside reverse transcriptase inhibitor in the same molecule. So the new



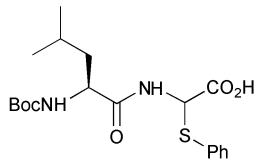
(191)



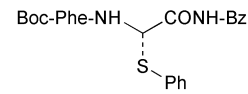
(192) $n = 3$, $m = 1$

hybrids bear structures such as (**192**), which turned out to be 920 and 62 times more potent than the inhibitor KN-727 and AZT respectively. Two synthons (**193**) and (**194**) where the methylene group of Phe has been replaced by sulfur atoms could be of use in inhibitor work.[490] Inhibition activity of $IC_{50} = 0.01$ μM gives promise[491] to a series of 6-hydroxy-1,3-dioxin-4-ones. The lead structure of palinavir has been further tuned[492] at the P3-P2 quinaldic-valine portion, to give as the most promising analogue (**195**) with an IC_{50} value of 1.6 nM and 61% bioavailability in rat. Exploration of the hydroxylamine-pentanamide transition-state isostere present in the drug indinavir sulfate has now yielded[493] a potent and selective analogue MK-944A (**196**) with a K_i value of 0.049 nM and a longer half-life in several animal models than indinavir, which has moved it on to advanced human clinical trials. Key syntheses of hydroxy ethylamine dipeptide isosteres in future will benefit from the stereoselective route worked out[494] for (**197**). Constrained dipeptidal sulfamides might represent novel peptidomimetic scaffolds in this area,[495] while the pseudo C_2-symmetric cyclic phosphonamides (**198**) were developed for their HIV-protease inhibition potential.[496]

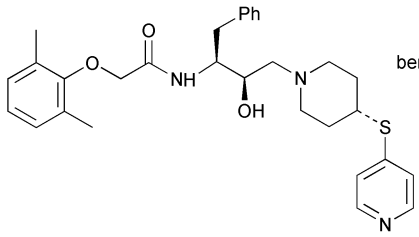
5.10 Matrix Metalloproteinase Inhibitors. – The use of matrix metalloproteinase (MMP) inhibitors for the treatment of cancer has been the subject of an annual report.[497] Functionalised tetrahydrofuran, such as (**199**) have been designed[498] as constrained inhibitors of stomelysin and related matrix metalloproteinases. The biological results were disappointing possibly due to the lack of H-bonding interactions. The Gly-Leu phosphinic dipeptide analogue, GlyΨ[PO_2H-CH_2]Leu has been chosen as the core of a 165 000 compound library,[499] and after analysis a consensus sequence of 20 analogues were re-synthesised. Inhibition down to 6 nM was achieved by some members, but only poor inhibition came from truncated sequences. Piperazine carboxylic acid has been used[500] as the core of a new generation of cyclic matrix metalloproteinase inhibitors. The bidentate chelating agent for Zn^{2+} is the hydroxamic acid group, and this together with the sulfonamide group produced the inhibitor (**200**), with high affinity for the matrix metalloproteinases-1, -3, -9 and -13. X-ray data for a complex between (**200**) and MMP-3 were also available. A pyrrolidinone scaffold has given rise to (**201**), a potent matrix MMP-13 inhibitor ($IC_{50} = 7$ nM).[501]



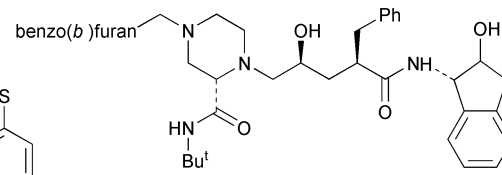
(193)



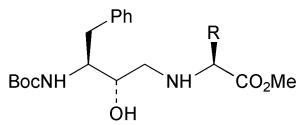
(194)



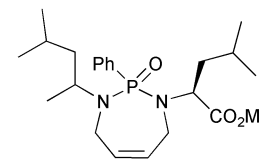
(195)



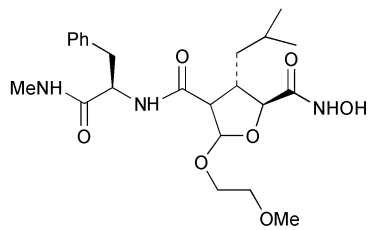
(196)



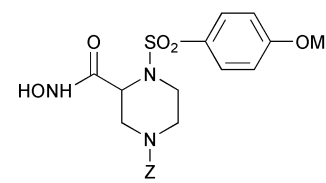
(197) R = $MeSCH_2CH_2$, $PhCH_2$, Me, Me_2CH or Me_2CHCH_2



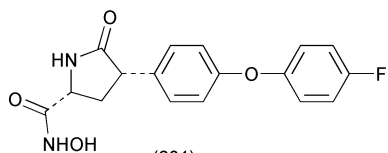
(198)



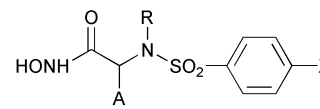
(199)



(200)



(201)



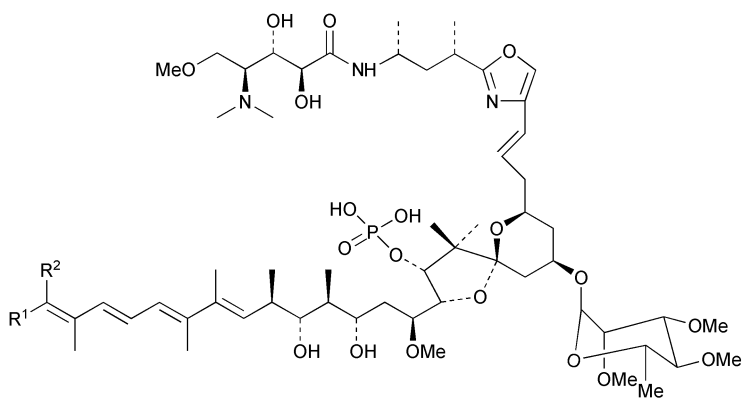
(202) A = H, Pr^i
R = Bu^i, Bn, *etc.*
X = OMe, Cl, H, Me, F, *etc.*

N-4-Nitrobenzylsulfonylglycine hydroxamate moieties such as (**202**) have undergone a series of assays with MMP enzymes.[502] Best inhibitors of MMP-1 were those involving fluorophenylsulfonyl moieties at P-1. Long perfluoroalkylsulfonyl moieties were preferred for the inhibition of the deep-pocket enzymes, MMP-2, -8 and -9.

Because the active geometry around the Zn ion in both the carbonic anhyd-

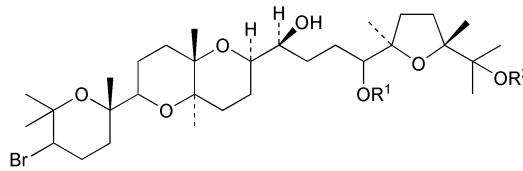
rase isozymes and matrix metalloproteinases are rather similar, an assessment[503] has been made as to the relative merits of sulfonylated amino acid hydroxamates as inhibitors in both series of enzymes. Core structures used were based on RSO_2NX-Aaa-CONHOH, with X = H, benzyl, substitiuted benzyl and Aaa representing Gly, Ala, Val or Leu. Out of the data it might be possible to design dual enzyme inhibitors with compact X (*e.g.* H) and Aaa (*e.g.* Gly) favouring carbonic anhydrase inhibition, wheras bulkier X and Aaa moieties and substituted aryl R groups are advantageous for showing better inhibition of MMP. Another sub-series of analogues on the same structural theme,[504] *e.g. N*-4-nitrobenzyl-β-alanine hydroxamates, have been assayed and confirm that the 4-nitrobenzyl group is an efficient P-2′ anchoring moiety. The tripeptide complex Gly-His-Lys-Cu^{2+}, a known wound healing agent, has been studied[505] for its effect on MMP-2 levels. It increased MMP-2 levels in cultured fibroblasts, but the effect is associated with the Cu ions. The peptide complex is able to modulate MMP expression by acting directly on wound ribroblasts.

5.11 Protein Phosphatase Inhibitors. – The mechanism of inhibition of protein phosphatase (PP) -1 by clavosines A (**203**) and B (**204**), novel members of the calyculin family of toxins has been investigated by site-directed mutagenesis.[506] Tyr^{134} is implicated as an important residue in PP-1cγ that mediates interactions with calyculins. Protein inhibitors I_1^{PP2A} and I_2^{PP2A} have been shown[507] to associate with and modify the substrate specificity of PP-1. The role of phosphorylated Thr^{35} and Ser^{67} in inhibitor I_1 protein has been investigated.[508] Protein kinase NCLK from brain extract was identified as a potential *in vivo* I_1 kinase, and that Thr^{35} and Ser^{67} phosphorylation independently activate I_1. Interaction of inhibitor 2 (I_2) potently inhibits the activity of the free catalytic sub-unit (CS 1) of PP-1, and the nature of this interaction has been fine-tuned[509] to a new model of interaction. The model involves multiple 'anchoring interactions' which serve to position a segment of I_2, such that it sterically occludes the catalytic pocket but need not make high affinity contacts itself. Backbone NMR resonances for inhibitor I_2 have been assigned.[510]



(203) R^1 = H, R^2 = $CONH_2$
(204) R^1 = $CONH_2$, R^2 = H

A convergent synthetic entry into the squalenoid polyether system has been developed[511] for the marine natural products (**205–208**) based on the thyrsiferyl moiety, to enable studies to be carried out on their PP 2A inhibitory activities. A targetted library of 70 compounds has been synthesised,[512] but only three of these



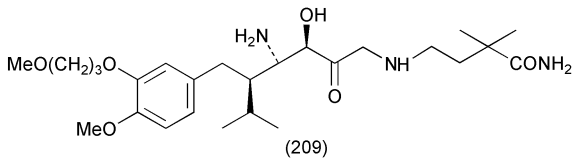
(205) R^1 = H, R^2 = Ac
(206) R^1 = H, R^2 = H
(207) R^1 = Ac, R^2 = H
(208) R^1 = Ac, R^2 = Ac

had ability to inhibit the dual specificity phosphatases Cdc25B(2) and none inhibited PTP1B. Three dual-specific phosphatases and three protein-tyrosine phosphatases have been challenged[513] with a set of low molecular weight *N*-(substituted)-*O*-phospho-L-serine derivatives. Only the dual-specific phosphatases, IphP and VHR hydrolysed the analogue *N*-(cyclohexanecarboxyl)-*O*-phospho-L-serine. It was already known that endothal had high affinity for PP2A. This molecule has now been modified[514] to 2-carboxymethylendothal to obtain improved affinity probes for PP2A. Information has been gathered[515] that PP2A and PI 3-kinase contribute to the ability of tumour antigen (small t) to regulate Spl activity, stimulate early SV40DNA replication, and enhance the transformation of resting cells during SV40 infection.

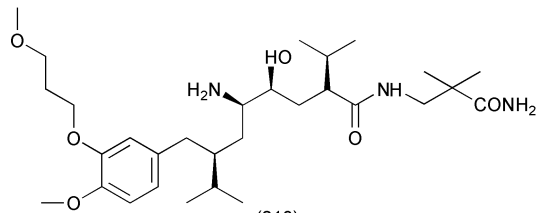
5.12 Renin and Other Aspartyl Proteinase Inhibitors. – The role of renin-angiotensin inhibitors as antihypertrophic agents has been highlighted.[516] An alternative convergent synthesis[517] to ones already published for renin inhibitor CGP 60536B (**209**) has appeared. The methodology is based on pseudoephedrine serving as a dual purpose chiral auxilliary and protecting group. Although the synthetic sequence involved 22 steps and an overall 7% yield a convergent stereoselective synthesis of the renin inhibitor BILA 2157 BS still produced 0.6 kg of the inhibitor.[518] After a detailed survey of six crystal structures of renin-inhibitor complexes, new inhibitors have been developed[519] with increasing lipophilic character replacing the peptide portion. Inhibitor (**210**) from this work had sub nanomolar binding affinity with a pronounced *in vivo* activity after oral administration to primates, making it a candidate for the clinic. The pharmokinetics of renin inhibitor CGP 60536 can now be assessed[520] *via* the development of a quantitiative hplc method for its analysis.

Exploration of active-site specificity of the aspartic proteinase, saccharopepsin, has been carried out[521] by investigating the X-ray structures of saccharopepsin with five renin inhibitors. The final stages in the generation of amyloid β-protein in Alzheimer's disease is proteolysis of the precursor protein with γ-secretase. Difluoro ketone peptidomimetics have been synthesised[522] to study

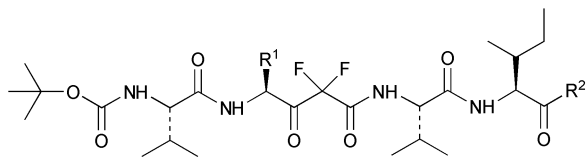
the inhibition of this enzyme and a series of inhibitors based on (**211**) have been assessed. Cyclohexyl methyl substituents in the P-1 position was useful for potency and indicates a large S-1 pocket to accommodate this bulkiness. The trifluoromethyl analogue of the aspartate protease inhibitor pepstatin has been synthesised,[523] but showed no anti-HIV activity.



(209)



(210)

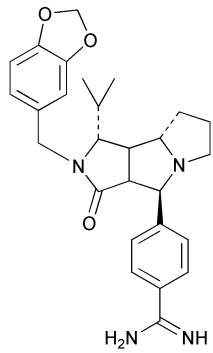


(211) R^1 = Me, Pr^i, cylohexylmethyl *etc.*
R^2 = OMe, OEt, NHMe, *etc.*

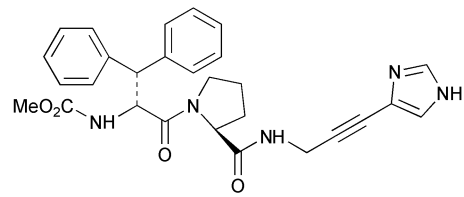
5.13 Thrombin and Factor Xa Inhibitors. – The challenge to obtain efficient inhibitors in this area remains very active. The computer programme GRID together with consensus component analysis (CPCA) has been used[524] to design selective ligands which have been validated on a total of nine structures of the three homologous serine proteases thrombin, trypsin and factor Xa. The rhynchobdellid leech *Theromyzon tessaulatum* yielded[525] the most potent thrombin inhibitor up to that time. Named, theromin, it contains 67 amino acids, including 16 cysteine residues, and exhibits no sequence homology with any other thrombin inhibitors. A series of thrombin inhibitors[526] based on (4-t-butylbenzenesulfonyl)-Arg-(D-Pipecolic acid)-Xaa-Gly-Yaa-Gly-β-Ala-Asp-Tyr-Glu-Pro-Glu-Glu-Ala-(β-cyclohexylalanine) have been prepared,[526] where the P1′ residue Xaa and the P3′ residue Yaa have been varied. D-PhGly or D-Phe in the Yaa position improved K_i values, compared to Gly. Substitution of Xaa with Nleu or L-β-(2-thienyl) alanine, lowered the K_i values, but when these residues were used with an improved linker, 12-aminododecanoic acid, they could be crystallised in complex with human α-thrombin to give a 2.1 Angstrom resolution structure, which showed that Lys^{60} in thrombin formed a large non-polar S1′ subsite to accommodate the bulky P1′ residue.

X-ray structural information on thrombin has guided[527] the structure-based

design of active and selective non-peptide inhibitors of the enzyme, which have side chains to reach the three binding pockets (selectivity S1 pocket, distal D pocket and proximal P pocket). The most potent in the series was found[528] to be (**212**) with a $K_i = 7$ nM. Amino acid residues in the thrombin-inhibiting tripeptide D-Phe-Pro-Arg have been replaced[529] with isosteres; Arg with *p*-amidinobenzylamine, Pro with either cyclopentane-1,2-carboxylic acid or cyclopentene-1,5-dicarboxylic acid and D-Phe with a series of lipophilic amines. Highly selective thrombin inhibitors with good oral absorption have been developed[530] by incorporating imidazolylethynyl groups into the P1 position as shown in (**213**). A bicyclic pyridone scaffold has been used[531] as a P3P2 surrogate to give inhibitors such as (**214**), and boron-containing inhibitors such as (**215**) and (**216**), although having side-chains too big for the S1 pocket, still inhibit[532] thrombin in the nM range. Non-amide based thrombin inhibitors such as (**217**)



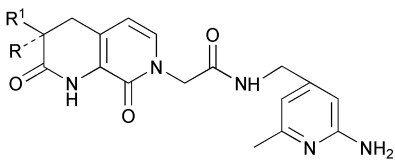
(212)



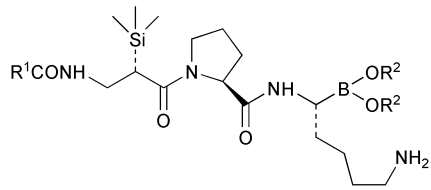
(213)

have been prepared[533] with amidinohydrazones as guanidine bioisoteres. This had a K_i value of 8.3 nM, while in another series such as (**218**), the optimal group for X to achieve nanomolar potency was the methyl group.[534] Exploration[535] of the S1 specificity pocket of thrombin gave (**219**) ($K_i = 69$ nM) as the most active compound in a series of inhibitors.

Non-polar substitutes for Arg in the P1 position have been used[536] in the development of analogues such as (**220**) of CGH-728, which is itself an analogue of Mitsibushi compound MD805. In a further optimisation[537] to improve water solubility, the R group has been modified as in (**221**), which is more bioavailable and shows efficacy in animal models of thrombosis. Two series of alkyl/aryl guanidines/sulfonyl *O*-methyl isoureas, based on the unit (**222**), have undergone a QSAR study[538] assayed against both trypsin and thrombin. The structural basis of thrombin/trypsin selectivity of an Arg mimic, (2*S*)-2-amino-(3*S*)-3-(1-carbamimidoyl-piperidin-3-yl) at the P1 position has been monitored[539] and it is believed that the selectivity achieved is mediated through differential interaction with the residue in position 192, Glu in the case of thrombin, Gln in trypsin. Diamino benzo[*b*]thiophene derivatives, such as (**223**) complexed with human α-thrombin, have given crystal structures[540] at 2.0 Angstrom resolution, which can enhance the understanding of the binding affinities. The results reveal unexpectedly that the benzothiophene nucleus of the inhibitor binds to the S-1

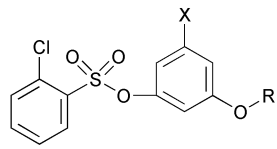


(214)

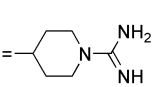


(215) $R^1 = Me_3COCO$, R^2R^2 =

(216) $R^1 = Me_2CHCH_2$, $R^2 = H$



(217) R =

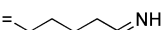


, X = Me

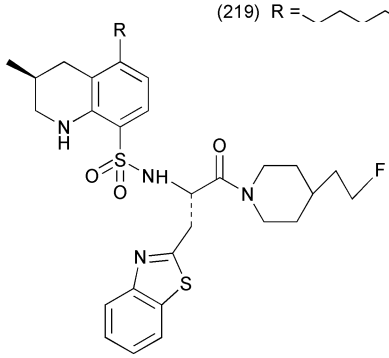
(218) R =

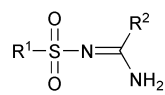
, X = Me

(219) R =



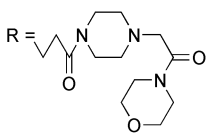
, X = Me

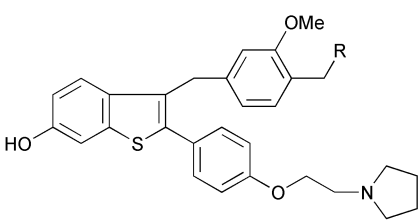




(222) R^1 = alkyl, aryl or aralkyl
R^2 = NHOMe

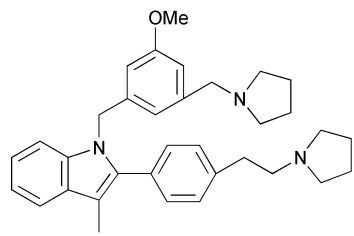
(220) R = H (221) R =





(223) R =

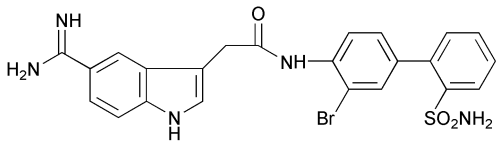
or



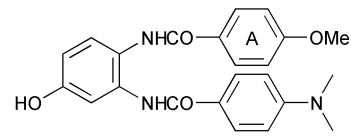
(224)

specific pocket, the basic C-3 side chain binds to the hydrophobic proximal S-2 and distal S-3 sites. The C-2 basic chain was found to point away from the active site. The most potent analogues,[541] such as (**223**), have been studied in an arterial/venous shunt model of thrombosis and were found to be efficacious in reducing clot formation, but had the disadvantage of rapid and extensive distribution after administration. Attempts[542] to improve the inhibitors have come from modifications to the C-4′ side chain and also through the preparation[543] of 1,2 -disubstituted indole, azaindole and benzimidazole derivatives. In this inhibitor series, indole-based inhibitor (**224**), with $K_{ass} = 197 \times 10^6\,L\,mol^{-1}$ proved to be the most potent. Several thrombin inhibitors have been prepared with MeArg in the P1′ amino acid position.[544] The most potent I-11 [D-Cha-Pro-MeArg-Thr Gly[5]-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-D-Glu] with a K_i of 3 pM shows stability in human plasma *in vitro*, with no inhibition of the related serine proteases, trypsin, factor Xa and plasmin. Phosphono tripeptides, such as *Z*-D-Dpa-Pro-OCHRP(O)$(OR')_2$ where Dpa is β,β-diphenylalanyl, and have an ester link between the P1 and P2 positions, inhibit thrombin in the micromolar range.[545] High levels of specificity for thrombin over other serine proteases have been achieved[546] in which a bicyclic lactam was used as the scaffold on which various P1 and P3 motifs were substituted. The subsequent K_i values were less than 10 nM, but the inhibitors showed low oral bioavailability, although showed promise when administered intravenously. Synthesis of P1 α-ketoamide protease inhibitors has been made easier[547] through the synthesis of α-hydroxy-β-aminoamides *via* the Passerini reaction.

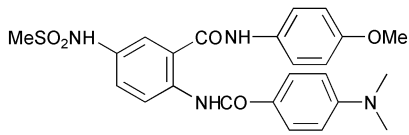
Factor Xa (Ma) plays a vital role in the regulation of normal homeostasis and abnormal intravascular thrombus development in the blood coagulation cascade. Hence it is an enzyme of great interest to the pharmaceutical industry. A novel series[548] of inhibitors of the enzyme have an amidino 6,5-fused bicyclic moieties at the P1 position and SAR studies show that the analogue (**225**) is the most potent in the series, with a K_i of 0.3 nM. Lead compounds derived from a combinatorial library of diamidophenols inhibit human factor Xa at micromolar concentrations.[549] The highest affinity factor Xa inhibitor in this series was (**226**). Reversal of the A-ring amide link has led to another series of inhibitors which could be further 'tuned' to give (**227**) as the best (K_i of 11.5 nM) in the series.[550] In a series of arginine aldehyde inhibitors designed[551] as transition state analogues based on a known factor Xa specific substrate, the most potent and selective was the aldehyde $BnSO_2$-D-Arg-Gly-Arg-H. Novel, potent and selective factor Xa inhibitors have evolved[552] from P1-ketoamide moieties. The top candidate within the series was (**228**) ($IC_{50} = 0.78$ nM). Further structural modification of conformationally restricted borolysine inhibitors of factor Xa has seen the boronic acid moiety replaced[553] by a-ketoamide, with compound (**229**) being chosen for further modelling. Reproducible crystallisation conditions have been found[554] for human des-Gla (1–45) coagulation fXa, with the result that crystal structures of complexes between the enzyme and three inhibitors have been elucidated at 2.3–2.8 Angstrom resolution.



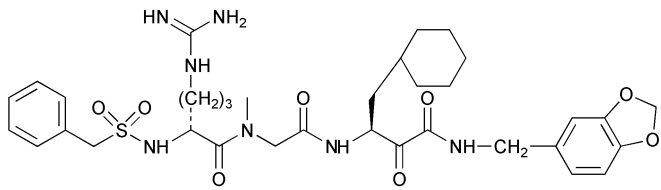
(225)



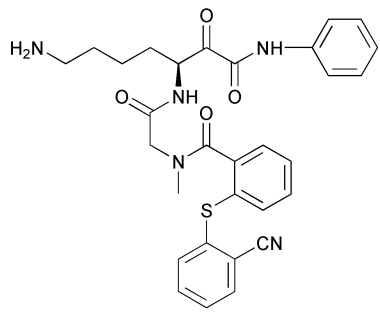
(226)



(227)



(228)



(229)

5.14 Proteinase-activated Receptors. – Proteinase-activated receptor-2 (PAR-2) is a member of a family of G-protein-coupled, seven-transmembrane domain receptors that are activated by proteolytic cleavage. Potential physiological activators identified so far include trypsin and mast cell tryptase (acrosin).[555] Cleavage of PAR-2, by unknown mechanisms, induce widespread inflammation. It is possible[556] that trypsin and tryptase directly signal to neurons to stimulate release of neuropeptides, which mediate inflammatory edema, induced by agonists of PAR-2. Thus tryptase inhibitors and antagonists of PAR-2 may be useful, anti-inflammatory agents. Further studies[557] confirm that pancreatic trypsin and trypsin-2 were almost equally effective at cleaving PAR-2, suggesting that extrapancreatic trypsins are potential *in vivo* activators of PAR-2. Activation of PAR-2 triggers mucin secretion in the rat sublingual gland[558] and can be attenuated by genistein, a tyrosine kinase inhibitor, but not by inhibitors of protein kinase C and phospatidyl inositol 3′-kinase. It has been found[559] that trypsin stimulates integrin-dependent adhesion and growth of MKN-1 human gastric

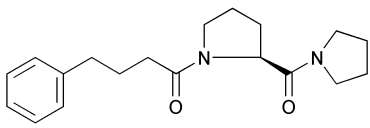
carcinoma cells. These latter cells expressed both PAR-1 and -2 which are activated by thrombin and trypsin respectively. Trypsin and the PAR-2 ligand stimulated growth of MKN-1 cells more strongly than thrombin or PAR-1, which resulted in the conclusion that trypsin regulates cell adhesion, and that integrin $\alpha_5\beta_1$ and integrin $\alpha_v\beta_3$-dependent cell adhesions are regulated by different PAR/G-protein signallings. As the evidence accumulates for the involvement of trypsin in stimulating divergent cellular reactions through PAR-2, the search for subtle inhibitors begins. In this context,[560] (2*R*,4*R*)-4-phenyl-1-[*N*-α-(7-methoxy-2-naphthalensulfonyl)-L-Arg]-2-piperidinecarboxylic acid shows promise in that it has a K_i value of 0.1 μM for trypsin, inhibited thrombin very weakly and did not inhibit plasmin, plasma kallikrein, urokinase or mast cell tryptase. The function of G-protein-coupled receptor kinases (GRKs) in the regulation of thrombin-activated signalling in endothelial cells has been studied,[561] and the results indicate the crucial role of GRK5 isoform in the mechanism of thrombin-induced desensitisation of PAR-1 in endothelial cells.

5.15 Miscellaneous. – N-(Hydroxyaminocarbonyl)-Phe-OH has been designed[562] as a new inhibitor of carboxypeptidase A. It is more potent as its D-Phe form (K_i = 1.54 μM) which is 3-fold more potent than the L-form.

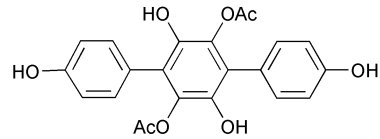
Dipeptidyl peptidase IV (DPP IV) has roles in T-cell costimulation, chemokine biology, type-II diabetes and tumour biology. It is a Pro-specific serine protease which cleaves Xaa-Pro dipeptides. A novel homologue of DPP IV, named DPP 8 has been cloned and expressed,[563] and shown to share postproline dipeptidyl aminopeptidase activity with DPP IV and FAP. Results suggest that there might be a role for DPP 8 in T-cell activation and immune function. To clarify the importance of the Cys residues, each of seven Cys residues in rat dipeptidyl II cDNA have been replaced using site-directed mutagenesis.[564] Six of the mutants had similar activity to that of the wild type but the ones with Cys^{176} replaced by either Ala, Gly or Glu showed no DPP LII activity, so Cys^{176} is essential for the regulation of DPP III activity. Natural substrates of DPP IV have been highlighted.[565] Potent inhibitors of DPP IV and their mechanism of inhibition have been investigated,[566] with the results showing that analogues of amino acid amides, bearing thioxo amide, phosphonoamide or reduced amide bonds function as classical reversible inhibitors of DPP IV, while amino acyl-2-cyanopyrrolidides inhibit DPP IV irreversibly with a slow binding mechanism giving inhibition constants in the nanomolar range. NMR spectroscopy and molecular dynamics calculations have been applied[567] to a series analogues of the N-terminal nonapeptide of the HIV-1 Tat protein which is a known inhibitor of DPP IV. Conformational backbone differences were revealed, as well as the arrangement of the side chains at significant positions. These have been used to explain why Ile^5-Leu^6 analogues are less inhibitory, while Gly^2 or Lys^2 analogues show considerably enhanced inhibition. Evidence has also been accumulated[568] that specific inhibitors of DPP IV suppress mRNA expression of DPP IV/CD26 and cytokines.

Prolylendopeptidase (PEP), a serine protease which cleaves peptides at the C-terminal side of Pro, is known[569] to be inhibited by SUAM-1221 (**230**). Further

fine tuning of this structure, with the assistance of comparative molecular field analysis (CoMFA), has shown that a six-methylene structure is optimal and replacing the benzene ring with *N*-heteroaromatics also improves inhibition. In the hope that PEP inhibitors might prevent memory loss and increase the attention span of patients, a search for natural inhibitors has revealed[570] that kynapcin (**231**) from *polyozellus multiplex* inhibits PEP. Screening[571] of fourteen traditional Kampo formulas for PEP inhibitors showed that Tokaku-joki-to shows a significant inhibitory activity. Along with a number of known carbohydrate derivatives extracted from the Kampo formula, *cis*-3,5,4′-trihydroxystilben 4′-*O*-β-D-(6-*O*-galloyl) glucopyranoside and 4-(4-hydroxyphenyl)-2-butanone 4′-*O*-β-D-(6-*O*-galloyl-2-*O*-cinnamoyl) glucopyranoside were new compounds each capable of inhibiting PEP.



(230)



(231)

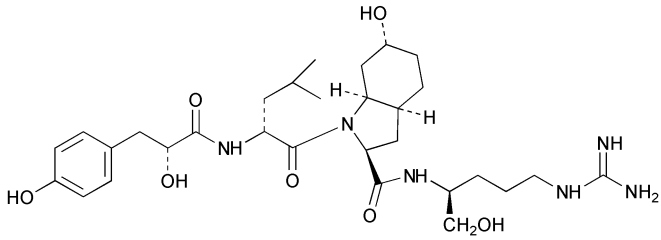
The protein tyrosine kinases (PTKs) catalyse the transfer of the gamma phosphate of ATP to tyrosine residues on protein substrates, which then creates binding sites for the recruitment of downstream signalling proteins. Important results that have emerged from high-resolution structural studies of PTKs and the mechanisms by which receptor and non-receptor PTKs are regulated have been reviewed.[572] Tyrosine substrate specificity within a short peptide has been investigated[573] for PTK Csk. Group substitiutions of the size of a methyl group or smaller can be tolerated on the Tyr aromatic ring, while the Tyr methylene group could be expanded by a single methylene and only the L-form of Tyr could be included. QSAR studies[574] on 104 flavanoid derivatives as p56(lck) PTK inhibitors have been performed using molecular descriptors calculated by CODESSA software. The immunoreceptor tyrosine-based activation motif (ITAM), mediates responses of proliferation and differentiation in transmembrane signal transduction in hematopoietic cells. Phosphorylation of the CD79a ITAM by Lyn, a Src family PTK is the initial signalling event, and to elucidate[575] the structural basis for recognition, exchange-transferred nuclear Overhauser NMR has been used. The data strongly favours ITAM binding in an orientation similar to binding cyclic AMP-dependent protein kinase rather than that of insulin receptor tyrosine kinase. When the PTK inhibitors, tyrphostins, are oxidised[576] with hypervalent iodine reagents, none of the oxidised products displayed enhanced activity *in vitro* in the NCI 60-cell line or in human breast cancer cell lines. The substrate selectivity of unnatural tyrosine derivatives for PTK Csk has been discussed.[577]

Inhibition of serine proteases is a very wide brief, but one review[578] has focussed on publications on the low molecular weight cyano peptide inhibitors. Three inducible serine protease inhibitors have been purified[579] from larval hemolymph of the greater wax moth *Galleria mellonella*. The inhibitors were identified as proteins ISPI-1, -2, and -3 with molecular masses of 9.2, 6.3 and 8.2

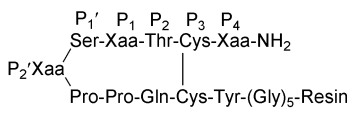
kDa respectively. The 3D structure of Apis mellifera chymotrypsin/cathepsin G inhibitor-1 (AMCI-1), from the honey bee hemolymph has been deduced from NMR data.[580] The 56-residue peptide has two approximately perpendicular β-sheets, several turns and a long exposed loop that includes the protease binding site. Stabilisation of the conformation is through five disulfide bridges. A total synthesis[581] and reassignment of configuration has been reported for aeruginosin 298-A (**232**), an active site serine protease inhibitor. The proteolytic sites of hepatitis C virus polyprotein have been probed using peptide aldehyde protease inhibitors. The peptide aldehydes have been synthesised[582] on resin using a previously described pro-aldehyde linker. Extrapolating from the capability of β-lactams to acylate the serine hydroxyl group in proteases, kinetic studies[583] have been carried out on model lactams with hydroxide. The study highlighted the as yet overlooked six-membered lactam ring as a promising vantage point for further development. A library of chymotrypsin inhibitors has been created[584] through the randomisation of the P_4 site and variation at positions P_1 and P'_2 in (**233**). The best K_i values were achieved when P_4, P_1 and P'_2 were either Nle, Phe and Ile, or Gln, Phe and Leu respectively. Parallel solution synthesis[585] of *N*-functionalised isatins has given products such as (**234**), which when screened against a panel of serine proteases gave high percentage inhibition.

A range of inhibitors of tissue factor/factorVIIa-induced coagulation have been based on the oxazine-4-one structure. In the 2-aryl substituted 4*H*-3,1-benzoxazin-4-one series (**235**), inhibition values[586] of between 0.17 to > 40 μM were achieved for compounds having one or two electronegative substituents representing R^2, while both electron attracting and donating groups could be accommodated for R^1. Several of the compounds showed a selectivity ratio towards factor X and thrombin of > 50. When ring A in (**235**) was replaced[587] by a series of heterocyclic rings the compounds gave a similar specificity as seen for the benzoxazine-4-ones. 6-(Aminomethyl)-5,6,7,8-tetrahydro-2-quinazolinamine and 4,5,6,7-tetrahydro-2*H*-indazol-5-ylmethamine have been synthesised[588] as conformationally restricted Arg side chain mimetics designed for incorporation into trypsin-like serine protease inhibitors. A P_1 Arg containing peptide library has been produced[589] through attaching the guanidinyl side chain to the polymer as seen in (**236**). Pro seemed preferrable in the P_2 position, but inactive in the P_3 position where a broad variety of residues could be tolerated. Hydrophobic residues did well in position P_4.

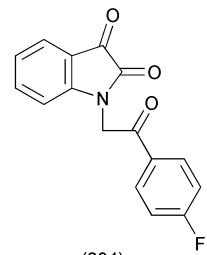
Human chymase, which converts angiotensins I to II with greater efficiency than ACE, participates in histamine release, activates precursor interleukin 1β and cleaves type I procollagen and progelatinase B, is speculated to have an important role in cardiovascular and chronic inflammation following fibrosis. Highly selective and potent inhibitors of the enzyme have come from SAR studies[590] on 1-oxacepham derivatives, with compound (**237**) proving the most potent. But its instability in human plasma had to be overcome by further studies[591] which gave (**238**) with a K_i value of 27 nM and a $t_{1/2}$ of 1.5 h in human plasma. An IC_{50} value of 0.36 μM for (**239**) was the best achieved[592] for a series of chloromethyl ketone inhibitors of human chymase.



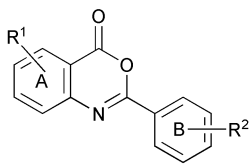
(232)



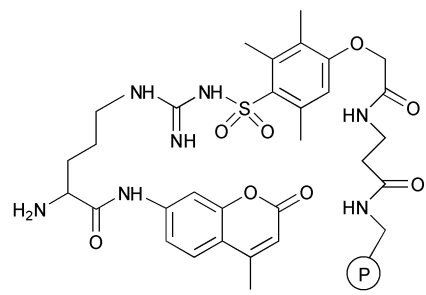
(233)



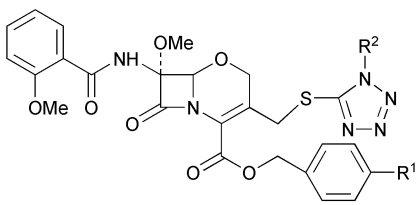
(234)



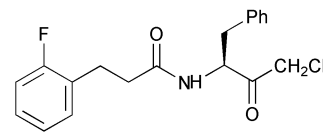
(235)



(236)



(237) R^1 = Me, R^2 = CH_2COOH
(238) R^1 = CO_2H, R^2 = Me

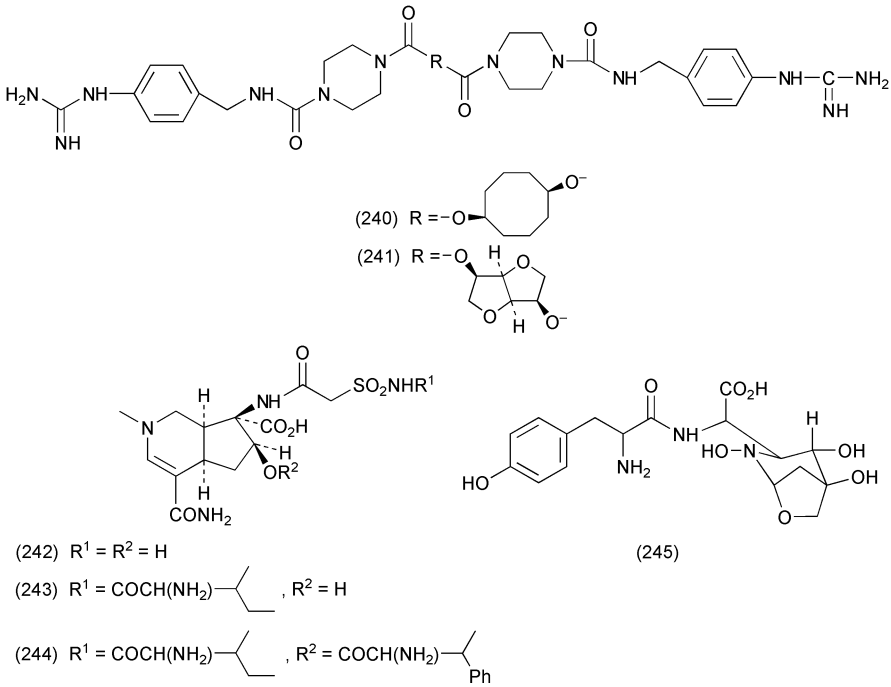


(239)

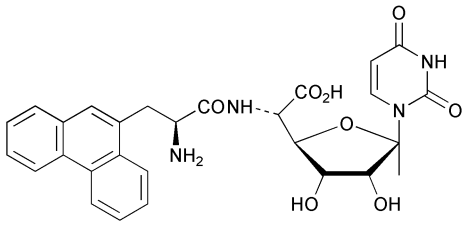
A second generation of reversible and active-site directed dibasic inhibitors of human mast cell tryptase have been developed,[593] the best amongst them being (**240**) with a K_i of 70 pM and a 10^5-fold selectivity versus anti-target proteases. Further optimisation[594] of the structure gave rise to APC-2059 (**241**), with sub-nanomolar activity, which has been advanced to phase II clinical trials for the treatement of psoriasis and ulcerative colitis.

A *Streptomyces* species has yielded[595] two isoleucyl tRNA synthetase inhibitors, SB 203207 (**243**) and SB-203208 (**244**) related to altemicidin (**242**), while a novel tyrosyl tRNA synthetase inhibtor, SB-219383 (**245**), has been isolated[596]

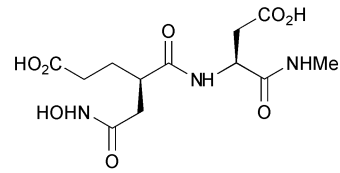
from a *Micromonospora* sp. Elaboration[597] of altemicidin has given (**243**), as well as 10 analogues. Substitution of the Ile residue of (**243**) with Leu and Val increased the inhibitor potency towards leucyl and valyl tRNA synthetases.



The final paragraph under this miscellaneous section highlights themes that our search has only generated one published paper for each in year 2000. A series of novel nikkomycin analogue inhibitors of chitin synthase have been synthesised.[598] Among them, compounds such as (**246**) having a phenanthrene group as N-terminal were found to have strong anti-chitin synthase activity. Inhibition of clostridium histolyticum collagenase, Chc, a zinc enzyme which degrades triple helical collagen can be inhibited[599] with sulfonylated L-valyl hydroxamates. The hydroxamates were generally 100–500-fold more active than corresponding carboxylates. Best substitution pattern within the sulfonyl moiety was perfluoroalkyl sulfonyl, substituted arylsulfonyl or 1- and 2-naphthyl. Cleavage of procollagen to collagen catalysed by procollagen C-proteinase can be inhibited by acidic dipeptide hydroxamates such as (**247**), which was the best ($IC_{50} = 10^{-7}$) inhibitor in the series.[600] By linking the N-termini of tripeptide aldehydes with a polymeric spacer, homo and heterobivalent inhibitors of eucaryotic proteosomes have been obtained.[601] An IC_{50} value of 0.017 μM was achieved by (**248**). In 1999 it was reported that the dipeptide amide Arg(NO_2)-L-Dbu-NH_2 ($K_i = 130$ nM) was the most potent and selective inhibitor of nitric oxide synthase. The original authors have now carried out SAR studies[602] to reveal the necessity for an α-NH_2 and an amide group joining the two amino acids. Proteosome inhibitors TMC-95A and B recently isolated (J. Kohno *et. al.*, *J. Org. Chem.*, 2000, **65**, 990) from

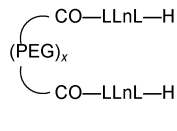


(246)

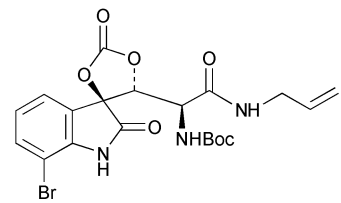


(247)

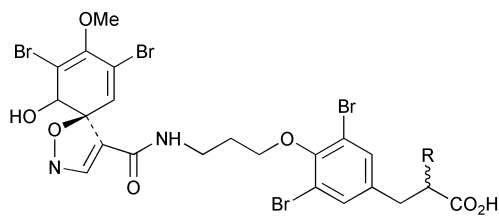
Apiospora montagnei Sacc have had the unit (**249**) moiety in their structure synthesised[603] from an oxindole and Garner aldehyde. Ianthesines A–D from an Australian marine sponge *Ianthella* sp. have been characterised[604] as A (**250**), B (**251**) and D (**252**) with C being a tetramer based on four dibromotyrosine units. Analogues B, C, and D showed Na, K-ATPase inhibitory activity in the range 50–440 μM whereas A is inactive.



(248)



(249)



(250) R = β-NMe_2
(251) R = α-NH_2
(252) R = $NHSO_3^-Na^+$

6 Phage Library Leads

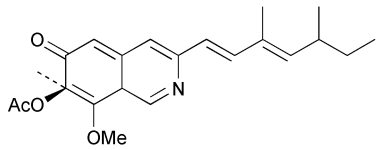
The term 'Biocombinatorial Chemistry' has been used[605] to define the rapid *in vitro* screening of molecular libraries, of which phage-displayed libraries is considered a representative method which is based on the affinity binding between displayed library and target molecule or tissue. In the examples reviewed, peptides have been created which mimic carbohydrate structures, thus giving rise to the term 'glyco-replica peptides'. A large synthetic antibody phage library has been panned[606] against biotinylated proteins secreted by normal human dermal fibroblasts, in the presence or in the absence of a molar excess of unbiotinylated proteins secreted by fibroblasts. Using MDCK cells[607] a polycationic peptide sequence, RYRGDLGRR, enhanced basal to apical transocyto-

sis of peptide-bearing phage 1000- to 10 000-fold compared with phage having no peptide insert. The synthetic peptides RYRGDLGRR and GRGDSP inhibited phage transcytosis implying the involvement of integrins. A recombinant mouse leptin[608] has been used to identify putative ligands using a phage library of random peptides. Three leptin-binding phage clones were found corresponding to peptide sequences, LAYCSDPVRCLVWWY, MFWISAVSFVDHALV and LVLVLSAFLCCGVG which could be useful in the study of leptin-receptor interaction, food intake and body-weight regulation. A phage-epitope library[609] has been used to identify peptide mimotopes capable of preventing the pathogenicity of the anti-MIR mAb 198. The peptide, PMTLPENYFSERPYH was found to bind specifically to mAb 198 and inhibited the binding to acetyl choline receptor. Cyclising the peptide *via* the insertion of extra residues and a pair of Cys residues each end gave a three orders of magnitude improvement in binding. A single chain Fv fragment 1F9 derived from a phage library[610] and complexed with turkey egg lysozyme has given a 2.0 Angstrom resolution X-ray structure, which has been used to explain differences in binding between the fragment when compared with egg-white lysozyme.

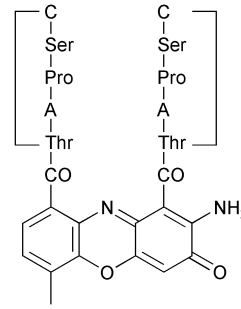
7 Protein–Protein Interaction Inhibitors

7.1 SH2 and SH3 Domain Ligands. – The rational design of small peptide inhibitors of Grb SH2 domain in the Ras signalling pathway and their pro-drug as antipoliferative agents have been discussed.[611] It has been observed that protein tyrosine kinases (PTKs) frequently contain SH2 domains which control signalling specificity. Substantial evidence[612] has been found for the existence of such a domain in Janus Kinases (JAKs), a family of PTKs, using web-based computational analysis in conjunction with preliminary binding studies in activated splenocytes.

8-*O*-Methylsclerotiorinamine (**253**) has been isolated[613] from a strain of *Penicillium multicolor*, and has been shown to significantly inhibit the binding between the Grb2-SH2 domain and the phosphopeptide from the Shc protein (IC_{50} = 5.3 μM). It also blocked the protein–protein interactions of Grb-Shc in cell-based experiments. During screening for Shc/Grb2 interactions it was discovered that actinomycins D, C2 and VII inhibit Grb2-SH2 domain binding. Analogues of actinomycin have now been synthesised and tested[614] and the best inhibitors turned out to be the Tyr-containing analogues (**254**) and (**255**), with IC_{50} values of 0.5 and 0.8 μM respectively. Analysis of X-ray crystal structures spawned[615] the idea that reducing the negative charge on a Tyr phosphate moiety by one could be compensated by an additional methylene group to create favourable interactions. In incorporating such phosphinate isosteres in a family of inhibitors, using 4-phosphonomethyl-phenylalanine derivatives[616] in solid phase protocols, the most active analogue was [**256**, R = PhCH(OH)] with an IC_{50} = 0.53 μM. Some of the tightest binding inhibitors known for the Src SH2 domain have come from the design and synthesis[617] of bicyclic non-peptide inhibitors such as (**257**) and (**258**). Stereochemistry was important in the binding



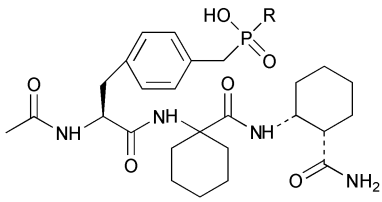
(253)



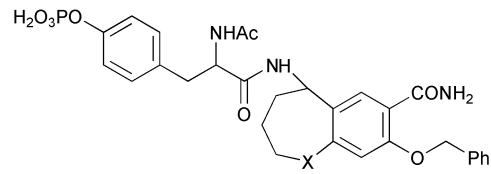
(254) A = D-Tyr, C = Val
(255) A = D-Val, C = D-Tyr

efficiences, with *SS* and *SR* forms of (**257**) having IC_{50} values of 0.2 and 1.6 μM respectively, and *SS* and *SR* forms of (**258**) recording values of 0.1 and 8.1 respectively. The idea of reducing the formal −2 charges on phosphotyrosine has also been explored[618] by the use of carboxymethyl-Tyr, carboxymethyl-Phe and carboxydifluoromethyl-Phe, and compared to their bis-carboxy-Tyr analogues, when incorporated into Ac-Asp-Ala-Asp-Glu-Xxx-Leu-NH_2. By assaying against Grb2 SH2 domains and against protein-tyrosine-phosphatase-1B (PTP1B), marked variation in relative potencies were discovered. Of particular note was the poor potency of all monocarboxy phosphotyrosine mimetics against PTP1B. An evaluation of the phosphotyrosine mimetic *p*-malonylphenylalanine has revealed[619] that, in Grb2 SH2 domain binding systems, the mimetic approaches the potency of phosphonate-base mimetics and is 15–20 times more potent than *O*-malonyltyrosine. A functionalised benzoic acid intermediate attached to a Rink amide link polymer support has generated a series of benzamide template-based non peptides for the exploration[620] of the hydrophobic binding at the pY + 3 pocket of Src SH2 domain. A non-phosphorylated cyclic peptide inhibitor, G1TE, of the Grb2 SH2 domain has been studied[621] in solution using NMR techniques. G1TE is made up of a decapeptide with a C-terminal Cys residue cyclised through its side chain by a thioether link to its N-terminus. A circle-like shape was detected with all side chains protruding on the outside, with no evidence of H-bonding and its restricted conformation might explain why it can compensate for the absence of a phosphate group at Tyr^3. The cytosolic tail of the mast cell function-associated antigen contains a Ser-Ile-Tyr-Ser-Thr-Leu sequence, which is a potential immunoreceptor tyrosine-based inhibition motif. To investigate[622] this further, peptides containing the 4–12 sequence of the mast cell function-associated antigen's N-terminal domain, containing the above motif, have been synthesised and used in affinity assays. Both phosphorylated and thiophosphorylated peptides bound to the Src SH2 domain. Neither non-phosphorylated, nor its tyrosyl phosphorylated reversed sequence analogue, bound to any of the phosphatases present in the domain. A report[623] has appeared detailing the large scale preparation of cell-permeable, non-phosphate containing Grb2 SH2 domain inhibitors.

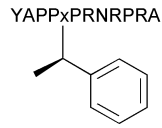
Src homology 3 (SH3) domains bind sequences bearing the consensus motif Pro-x-x Pro where x is any amino acid, with domain specificity mediated by



(256)



(257) X = O
(258) X = CH_2



(259)

sequences flanking this tetrapeptide core. A new source of ligand specificity has now been made possible[624] through the use of N-substituted residues (peptoids) instead of Pro in the motif. The best example synthesised was compound (**259**) which had K_{act} of 7.9 μM which compared well with the 'wild type' sequence YEVPPPVPPRRR which had a value of 88 μM. NMR methods have been developed[625] to monitor ligand-induced changes in H-bond geometry in the chicken c-Src SH3 domain. Key to the method was the measurement of *trans*-H bond ^{15}N-^{13}C scalar couplings in the free state and when bound to the high affinity class I ligand RLP2 containing residues RALPPLPRY. The results were in good agreement with X-ray data. Two backbone to side chain H-bonds are observed in solution, both appearing to stabilise the loop structure. Upon ligand binding, mutual intercalation of the two Leu-Pro segments of the ligand, between three aromatic side chains protruding from the SH3 surface wedges, impart secondary structural elements within the SH3 domain. A fully ^{13}C-^{15}N labelled and a selectively-labelled $^{13}C(\beta)$-Ala, ^{15}N(ring)Trp sample of SH3 domain of chicken α-spectrin, a 62-residue protein, have been biosynthesised[626] and studied by solid phase (Magic Angle Spinning) NMR. Spectral editing with the TEDOR technique gave a drastic simplification of the ^{13}C spectra, which allowed distances to be measured between residues Ala[55] and Trp[42], and compared with X-ray data.

8 References

1. A. S. Dutta in *Specialist Periodical Reports*, *Amino Acids*, *Peptides and Proteins*, 2001, **32**, pp. 163–286.
2. C. A. Selects in *Amino Acids*, *Peptides and Proteins*, published by the American Chemical Society and Chemical Abstracts Service, Columbus, Ohio.
3. *The ISI Web of Science Service for UK Education* on http://wos.mimas.ac.uk.
4. *Methods in Molecular Medicine No* 23 (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999.
5. Tetrahedron Symposia in Print No 83, *Tetrahedron*, 2000, Issue No 50.
6. J. H. Jones, *J. Pept. Sci*., 2000, **6**, 201.

7. M. Liley and T. Johnson, *Tetrahedron Lett*., 2000, **41**, 3983.
8. M. Falorni, G. Giacomelli, A. Porcheddu and G. Dettori, *Eur. J. Org. Chem*., 2000, 3217.
9. J. Cesar and M. Sollner, *Synth. Commun*., 2000, **30**, 4147.
10. K. Luthman, S. Borg and U. Hacksell in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 1.
11. J. Zabrocki and G. R. Marshall in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 417.
12. T. C. Henninger and P. Wipf in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 125.
13. B. S. Fulton, A. P. Kaplan Jr. and S. L. Gallion in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 527.
14. A. Scott, B. G. Conway and M. A. Krook in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 437.
15. G. Pesenti, A. Arnone, A. M. Aubertin, P. Bravo, M. Frigerio, W. Panzeri, S. Schmidt, F. Viani and M. Zanda, *Tetrahedron Lett*., 2000, **41**, 7239.
16. M. P. Dwyer and S. F. Martin in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 407.
17. W. M. Kazmierski and P. C. Fritch in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 281.
18. J. T. Welch and T. Allmendinger in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 357.
19. A. Volonterio, P. Bravo and M. Zanda, *Org. Lett*., 2000, **2**, 1827; A. Volonterio, P. Bravo and N. Moussier, *Tetrahedron Lett*., 2000, **41**, 6517.
20. D. Besser, B. Müller, I. Agricola and S. Reissmann, *J. Pept. Sci*., 2000, **6**, 130.
21. C. Schumann, L. Seyfarth, G. Greiner and S. Reissmann, *J. Pept. Res*., 2000, **55**, 428.
22. A. Dal Pozzo, N. Fagnoni, R. Bergonzi, L. Vanini, R. de Castiglione, C. Aglio and S. Colli, *J. Pept. Res*., 2000, **55**, 447.
23. A. Calcagni, E. Gavuzzo, G. Lucente, F. Mazza, E. Morera, M. P. Paradisi and D. Rossi, *Biopolymers*, 2000, **54**, 379.
24. M. C. F. Mornee, M. F. Marijne, A. J. Brouwer and R. M. J. Liskamp, *Tetrahedron Lett*., 2000, **41**, 7991.
25. J. C. Marsters Jr. and T. E. Rawson in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 385.
26. T. Sifferlen, M. Rueping, K. Gademann, B. Jaun and D. Seebach, *Helv. Chim. Acta*, 1999, **82**, 2067.
27. J. A. Robinson, *Synlett*, 2000, 429.
28. S. A. Andronati, A. A. Krysko, V. M. Kabanov, T. L. Karaseva and T. A. Kabanova, *Acta Pol. Pharm*., 2000, **57**, 15.
29. Y. Le Merrer, L. Poitout and J.-C. Depezay in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 227.
30. J. Brask and K. J. Jensen, *J. Pept. Sci*., 2000, **6**, 290.
31. G. Müller, G. Hessler and H. Y. Decornez, *Angew. Chem. Int. Ed*., 2000, **39**, 894.
32. L. Belvisi, C. Gennari, A. Madder, A. Mielgo, D. Potenza and C. Scolastico, *Eur. J. Org. Chem*., 2000, 695.
33. J. Mulzer, F. Schülzchen and J.-W. Bats, *Tetrahedron*, 2000, **56**, 4289.
34. M. E. Pfeifer, K. Moehle, A. Linden and J. A. Robinson, *Helv. Chim. Acta*, 2000, **83**, 444.
35. A. Golebiowski, S. R. Klopfenstein, J. J. Chen and X. Shao, *Tetrahedron Lett*., 2000, **41**, 4841.

36. Y. M. Fobian and K. B. Moeller, in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 259.
37. F. Rubsam, R. Mazitschek and A. Giannis, *Tetrahedron*, 2000, **56**, 8481.
38. A. Golebiowski, S. R. Klopfenstein, X. Shao, J. J. Chen, A.-O. Colson, A. L. Grieb and A. F. Russell, *Org. Lett*., 2000, **2**, 2615.
39. U. Bhatt and G. Just, *Helv. Chim. Acta*, 2000, **83**, 722.
40. Y. Tong, J. Olczak, J. Zabrocki, M. C. Gershengorn, G. R. Marshall and K. D. Moeller, *Tetrahedron*, 2000, **56**, 9791.
41. D. S. Perlow and R. M. Friedinger in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 209.
42. J. E. Davies and A. B. Holmes, *J. Chem. Soc. Perkin Trans. 1*, 2000, 2943.
43. S. Derrer, J. E. Davies and A. B. Holmes, *J. Chem. Soc. Perkin Trans. 1*, 2000, 2957.
44. D. Kaiser, G. Videnov, C. Maichle-Mössmer, J. Strähle and G. Jung, *J. Chem. Soc. Perkin Trans. 2*, 2000, 1081.
45. R. C. F. Jones and J. Dickson, *J. Pept. Sci*., 2000, **6**, 621.
46. A. B. Smith III, W. Wang, P. A. Sprengeler and R. Hirschmann, *J. Am. Chem. Soc*., 2000, **122**, 11037.
47. K. Burgess and W. Li in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 25.
48. A. I. Jiménez, C. Cativiela and M. Marraud, *Tetrahedron Lett*., 2000, **41**, 5353.
49. J. P. Davidson and S. F. Martin, *Tetrahedron Lett*., 2000, **41**, 9459.
50. D. Tourwe, K. Iterbeke, W. M. Kazmierski and G. Toth in *Methods in Molecular Medicine* (*Peptidomimetic Protocols*) ed. W. M. Kazmierski, Humana Press, 1999, **23**, 321.
51. S. Kotha, K. Mohanraja and S. Durani, *Chem. Commun*. (*Cambridge*), 2000, 1909.
52. X. Li, L. Zhang, W. Zhang, S. E. Hall and J. P. Tam, *Org. Lett*., 2000, **2**, 3075.
53. B. Westermann, N. Diedrichs, I. Gedrath and A Walter in *Biorg. Chem*., ed. U. Diederischen, Wiley-VCH Verlag, Weinheim, 1999, p. 188.
54. G. Suter, S. A. Stoykova, A. Linden and H. Heimgartner, *Helv. Chim. Acta*, 2000, **83**, 2961.
55. M. Saviano, R. Iacovino, V. Menchise, E. Benedetti, G. M. Bonara, M. Gatos, L. Graci, F. Formaggio, M. Crisma and C. Toniolo, *Biopolymers*, 2000, **53**, 200.
56. E. Medina, A. Moyano, M. A. Pericas and A. Riera, *Helv. Chim. Acta*, 2000, **83**, 972.
57. M. MacDonald, D. V. Velde and J. Aube, *Org. Lett*., 2000, **2**, 1653.
58. D. Giannotti, E. Perrotta, C. Di Bugno, R. Nannicini, N. J. S. Harmat, A. Giolitti, R. Patachini, A. R. Renzetti, L. Rotondaro, S. Guiliani, M. Altamura and C. A. Maggi, *J. Med. Chem*., 2000, **43**, 4041.
59. M. Rueping, B. Jaun and D. Seebach, *Chem. Commun*. (*Cambridge*), 2000, 2267.
60. T. Zoller, J.-B. Ducep, C. Tahtaoui and M. Hibert, *Tetrahedron Lett*., 2000, **41**, 9989.
61. T. J. Baker, Y. Rew and M. Goodman, *Pure Appl. Chem*., 2000, **72**, 347.
62. H. Li, X. Jiang, S. B. Howell and M. Goodman, *J. Pept. Sci*., 2000, **6**, 26.
63. H. E. Garay, O. Niebla, L. J. Gonzalez, T. Menendez, L. J. Cruz and O. Reyes, *Lett. Pept. Sci*., 2000, **7**, 97.
64. A. Cuthbertson and I. Indrevoll, *Tetrahedron Lett*., 2000, **41**, 3661.
65. E. Ogru, J. C. Wilson, M. Heffernan *et al*., *J. Pept. Res*., 2000, **56**, 388.
66. Y. Takebayashi, H. Koga, J. Togami, A Inui, H. Kurihara, K. Koshiya, T. Furuya, A. Tanaka and K. Murase, *J. Pept. Res*., 2000, **56**, 409.
67. T. Inui, M. Nakao, H. Nishio, Y. Nishiuchi, S. Kojima, T. Muramatsu and T. Kimura, *J. Pept. Res*., 2000, **55**, 384.

68. E. Kudryavtseva, M. V. Sidorova, M. V. Ovchinnikov and Z. D. Bespalova, *J. Pept. Sci.*, 2000, **6**, 208.
69. R. Söll and A. G. Beck-Sickinger, *J. Pept. Sci.*, 2000, **6**, 387.
70. K. Hashimoto, T. Okuno, M. Nakata and H. Shirahama, *Curr. Top. Phytochem.*, 2000, **3**, 1.
71. I. Wirkus-Romanowska, S. Rodziewicz-Motowildo, H. Miecznikowska, K. Rolka, M. Janusz, S. Szymaniec, A. Zablocka, W. Fortuna, R. Miedgybrodzki, J. Liskowski and G. Kupryszewski, *Pol. J. Chem.*, 2000, **74**, 1129.
72. J. P. Greenfield, R. S. Gross, G. K. Gouras and H. X. Xu, *Frontiers in Bioscience*, 2000, **5**, D 72.
73. J. Jarvet, P. Domberg, K. Bodell, L. E. Göran Eriksson and A. Gräslund, *J. Am. Chem. Soc.*, 2000, **122**, 4261.
74. B. M. Austen, E. R. Frears and H. Davies, *J. Pept. Sci.*, 2000, **6**, 459.
75. Y. Takahashi, T. Yamashita, A. Ueno and H. Mihara, *Tetrahedron*, 2000, **56**, 7011.
76. S. E. Blondelle and K. Lohner, *Biopolymers*, 2000, **55**, 74.
77. M. J. van Belkum and M. E. Stiles, *Nat. Prod. Reports*, 2000, **17**, 323.
78. I. F. Nes and H. Hole, *Biopolymers*, 2000, **55**, 50.
79. L. Otvos, *J. Pept. Sci.*, 2000, **6**, 497.
80. L. Otvos, M. E. Rogers, P. J. Consolvo, B. A. Condie, S. Lovas, P. Bulet and M. Blaszcyk-Thurin, *Biochemistry*, 2000, **39**, 14150.
81. L. Otvos, K. Bokonyi, I. Varga, B. I. Otvos, R. Hoffmann, H. C. J. Ertl, J. D. Wade, A. M. McManus, D. Craik and P. Bulet, *Protein Sci.*, 2000, **9**, 742.
82. N. F. Dawson, D. J. Craik, A. M. McManus, S. G. Dashper, E. C. Reynolds, G. W. Tregear, L. Otvos and J. D. Wade, *J. Pept. Sci.*, 2000, **6**, 19.
83. Y. S. Yang, G. Mitta, A. Chavanieu, B. Calas, J. F. Sanchez, P. Roch and A. Aumelas, *Biochemistry*, 2000, **39**, 14436.
84. S.-U. Oh, S.-J. Lee, J.-H. Kim and I.-D. Yoo, *Tetrahedron Lett.*, 2000, **41**, 61.
85. J. Jaworski and H. Brückner, *J. Pept. Sci.*, 2000, **6**, 149.
86. S. Rebuffat, C. Goulard, S. Hlimi and B. Bodo, *J. Pept. Sci.*, 2000, **6**, 519.
87. C. Peggion, C. Piazza, F. Formaggio, M. Crisma, C. Toniolo, B. Kaptein, Q. B. Broxterman and J. Kamphius, *Lett. Pept. Sci.*, 2000, **7**, 9.
88. T. Rozek, K. L. Wegener, J. H. Bowie, I. N. Olver, J. A. Carver, J. C. Wallace and M. J. Tyler, *Eur. J. Biochem.*, 2000, **267**, 5330.
89. B. C. S. Chia, Y. H. Lam, M. Dyall-Smith, F. Separovic and J. H. Bowie, *Lett. Pept. Sci.*, 2000, **7**, 151.
90. L. Silvestro and P. H. Axelsen, *Biophys. J.* 2000, **79**, 1465.
91. N. Ebran, S. Julien, N. Orange, B. Auperin and G. Molle, *Biochim. Biophys. Acta, Biomembranes*, 2000, **1467**, 271.
92. S. Kobayashi, K. Takeshima, C. B. Park, S. C. Kim and K. Matsuzaki, *Biochemistry*, 2000, **39**, 8648.
93. C. B. Park, K. S. Yi, K. Matsuzaki, M. S. Kim and S. C. Kim, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 8245.
94. D. Oh, S. Y. Shin, S. Lee, J. H. Kang, S. D. Kim, P. D. Ryu, K. S. Hahm and Y. Kim, *Biochemistry*, 2000, **39**, 11855.
95. S. Y. Shin, J. H. Kang, S. Y. Jang, Y. Kim, K. L. Kim and K. S. Hahm, *Biochim. Biophys. Acta, Biomembranes*, 2000, **1463**, 209.
96. A. Torres-Larios, G. B. Gurrola, F. Z. Zamudio and L. D. Possani, *Eur. J. of Biochem.*, 2000, **267**, 5023.
97. S. Y. Shin, S. W. Kang, D. G. Lee, S. H. Eom, W. K. Song and J. I. Kim, *Biochem. Biophys. Res. Commun.*, 2000, **275**, 904.

98. C. Gonzalez, G. M. Langdon, M. Bruix, A. Galvez, E. Valdivia, M. Maqueda and M. Rico, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 11221.
99. M. Azuma, T. Kojima, I. Yokoyama, H. Tajiri, Y. Yoshikawa, S. Saga and C. A. Del Capio, *Peptides*, 2000, **21**, 327.
100. M. B. Strom, O. Rekdal and J. S. Svendsen, *J. Pept. Res.*, 2000, **56**, 265.
101. F. Kasprzykowski, C. Schalen, R. Kasprzykowska, B. Jastrzebska and A. Grubb, *APMIS*, **108**, 473.
102. Z. Oren and Y. Shai, *Biochemistry*, 2000, **39**, 6103.
103. S. Burrage, T. Raynham, G. Williams, J. W. Essex, C. Allen, M. Cardno, V. Swali and M. Bradley, *Chem. Eur. J.*, 2000, **6**, 1455.
104. S. Roychoudhury, S. E. Blondelle, S. M. Collins, M. C. Davis, H. D. McKeever, R. A. Houghten and C. N. Parker, *Mol. Diversity* 1998 (publ. 2000), **4**, 173.
105. A. J. De Lucca, *Expert Opinion on Investigational Drugs*, 2000, **9**, 273.
106. M. H. Metz-Boutigue, K. Lugardon, Y. Goumon, R. Raffner, J. M. Strub and D. Aunis, *Adv. Exp. Med. Biol.*, 2000, **482**, 299.
107. J. Jamison, S. Levy, X. Sun, D. Zeckner, W. Current, M. Zweifel, M. Rodriguez, W. Turner and S.-H. Chen, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2101.
108. S.-H. Chen, X. Sun, J. Paschal, D. Zeckner, W. Current, M. Zweifel and M. Rodriguez, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2107.
109. B. Lopez-Garcia, L. Gonzalez-Candelas, E. Perez-Paya and J. F. Marcos, *Mol. Plant-Microbe Interactions*, 2000, **13**, 837.
110. B. Kundu, S. K. Rastogi, S. Batra, S. K. Raghuwanshi and P. K. Shukla, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1779.
111. P. Gavit and M. Better, *J. Biotech.*, 2000, **79**, 127.
112. F. L. Strand, *Eur. J. Pharmacol.*, 2000, **405**, 3.
113. J. Matsoukas, L. Polevaya, J. Ancans, T. Mavromoustakos, A. Kolocouris, P. Roumelioti, D. V. Vlahakos, R. Yamdagni, Q. Wu and G. J. Moore, *Bioorg. Med. Chem.*, 2000, **8**, 1.
114. J. Perodin, R. Bouley, E. Escher, N. Assimomytis, V. Magafa, E. Manessi-Zoupa, D. Theodoropoulos and P. Cordopatis, *Arzmittel-Forschung-Drug Res.*, 2000, **50**, 526.
115. C. Oiry, J. Pannequin, N. Bernad, A. M. Artis, J. C. Galleyrand, C. Devin, M. Cristau, J. A. Fehrentz and J. Martinez, *Eur. J. Pharmacology*, 2000, **403**, 17.
116. M. Casibang and T. W. Moody, *Peptides*, 2000, **21**, 649.
117. M. Cristau, C. Devin, C. Oiry, O. Chaloin, M. Amblard, N. Bernad, A. Heitz, J.-A. Fehrentz and J. Martinez, *J. Med. Chem.*, 2000, **43**, 2356.
118. K. S. Lin, K. E. Baidoo, H. Hashemzadeh-Gargari and H. N. Wagner, *J. Nucl. Med.*, 2000, **41**, 155.
119. V. Martinez and Y. Tache, *Peptides*, 2000, **21**, 1617.
120. A. Cullen, R. L. Emanuel, J. S. Torday, N. Asokananthan, K. A. Sikorski and M. E. Sunday, *Peptides*, 2000, **21**, 1627.
121. H. Ohki-Hamazaki, *Prog. in Neurobiol.*, 2000, **62**, 297.
122. T. W. Moody, R. T. Jensen, L. Garcia and J. Leyton, *Eur. J. Pharmacology*, 2000, **409**, 133.
123. N. Sakura, K. Kurosawa and T. Hashimoto, *Chem. Pharm. Bull.*, 2000, **48**, 1166.
124. A. L. Salmon, A. H. Johnsen, M. Bienert, G. McMurray, K. A. Nandha, S. R. Bloom and C. Shaw, *J. Biol. Chem.*, 2000, **275**, 4549.
125. Y. J. Basir, F. C. Knoop, J. Dulka and J. M. Conlon, *Biochim. Biophys. Acta, Protein Struct. and Mol. Enzymology*, 2000, **1543**, 95.
126. P.G. Szekeres, A. I. Muir, L. D. Spinage, J. E. Miller, S. I. Butler, A. Smith, G. I. Rennie, P. R. Murdock, L. R. Fitzgerald, H. L. Wu, L. J. McMillan, S. Guerrera, L.

Vawter, N. A. Elshourbagy, J. L. Mooney, D. J. Bergsma, S. Wilson and J. K. Chambers, *J. Biol. Chem.*, 2000, **275**, 20247.
127. M. Hosoya, T. Moriya, Y. Kawamata, S. Ohkubo, R. Fujii, H. Matsui, Y. Shintani, S. Fukusumi, Y. Habata, S. Hinuma, H. Onda, O. Nishimura and M. Fujino, *J. Biol. Chem.*, 2000, **275**, 29528.
128. M. G. Bock and J. Longmore, *Curr. Opin. Chem. Biol.*, 2000, **4**, 401.
129. M. Amblard, P. Bedos, C. Olivier, I. Daffix, J.-M. Luccarini, P. Dodey, D. Pruneau, J.-L. Paquet and J. Martinez, *J. Med. Chem.*, 2000, **43**, 2382.
130. P. Bedos, M. Amblard, G. Subra, P. Dodey, J.-M. Luccarini, J.-L. Paquet, D. Prumneau, A. Aumelas and J. Martinez, *J. Med. Chem.*, 2000, **43**, 2387.
131. S. Meini, R. Patacchini, A. Lecci, L. Quartara and C. A. Maggi, *Eur. J. Pharmacol.*, 2000, **409**, 185.
132. H. I. Trzeciak, W. Kozik, S. Melhem, A. Kania, D. Dobrowolski, A. Prahl, I. Derdowska and B. Lammek, *Peptides*, 2000, **21**, 829.
133. R. A. Lew, E. Boulos, K. M. Stewart, P. Perlmutter, M. F. Harte, S. Bond, M.-I. Aguilar and A. I. Smith, *J. Pept. Sci.*, 2000, **6**, 440.
134. L. F. Pineda, C. Liebmann, S. Hensellek, I. Paegelow, T. Steinmetzer, A. Schweinitz, J. Sturzebecher and S. Reissmann, *Lett. Pept. Sci.*, 2000, **7**, 69.
135. C. Schumann, L. Seyfarth, G. Greiner and S. Reissmann, *J. Pept. Res.*, 2000, **55**, 428.
136. M. Gobbo, L. Biondi, F. Filira, R. Rocchi and T. Piek, *Lett. Pept. Sci.*, 2000, **7**, 171.
137. Y. J. Basir, F. C. Knoop, J. Dulka and J. M. Conlon, *Biochim. Biophys. Acta, Protein Struct. Mol Enz.*, 2000, **1543**, 95.
138. M. Miskolie, H. Yamamoto, E. J. York, J. M. Stewart and G. Kotovych, *J. Biomol. Struct. Dyn.*, 2000, **17**, 947.
139. M. Miskolzie, L. Gera, J. M. Stewart and G. Kotovych, *J. Biomol. Struct. Dyn.*, 2000, **18**, 249.
140. K. Krause, L. F. Pireda, R. Peteranderl and S. Reissmann, *J. Pept. Res.*, 2000, **55**, 63.
141. P. B. Simpson, A. J. Wollacott, R. G. Hill and G. R. Seabrook, *Eur. J. Pharmacol.*, 2000, **392**, 1.
142. R. F. Turchiello, L. Juliano, A. S. Ito and M. T. Lamy-Freund, *Biopolymers*, 2000, **54**, 211.
143. G. Wotherspoon and J. Winter, *Neuroscience Lett.*, 2000, **294**, 175.
144. M. E. Pierson, J. M. Comstock, R. D. Simmons, R. Julien, F. Kaiser and J. D. Rosamond, *J. Med. Chem.*, 2000, **43**, 2350.
145. E. J. Verspohl and M. LaMura, *Hormone Res.*, 2000, **53**, 177.
146. B. Bellier, M.-E. Million, S. DaNascimento, H. Meudal, S. Kellou, B. Maigret and C. Garbay, *J. Med. Chem.*, 2000, **43**, 3614.
147. D. Vishnuvardhan and M. C. Beinfeld, *Biochemistry*, 2000, **39**, 13825.
148. C. M. R. Low, J. W. Black, H. B. Broughton, I. M. Buck, J. M. R. Davies, D. J. Dunstone, R. A. D. Hull, S. B. Kalindjian, I. M. McDonald, M. J. Pether, N. P. Shankley and K. I. M. Steel, *J. Med. Chem.*, 2000, **43**, 3505.
149. I. M. McDonald, D. J. Dunstone, S. B. Kalindjian, I. D. Linney, C. M. R. Low, M. J. Pether, K. I. M. Steel, M. J. Tozer and J. G. Vinter, *J. Med. Chem.*, 2000, **43**, 3518.
150. A. Ursini, A. M. Capelli, R. A. E. Carr, P. Cassara, M. Corsi, O. Curcuruto, G. Curotto, M. D. Cin, S. Davalli, D. Donati, A. Feriani, H. Finch, G. Finizia, G. Gaviraghi, M. Marien, G. Pentassuglia, S. Polinelli, E. Ratti, A. Reggiani, G. Tarzia, G. Tedesco, M. E. Tranquillini, D. G. Trist and F. T. M. van Amsterdam, *J. Med. Chem.*, 2000, **43**, 3596.
151. N. Bernad, B. G. M. Burgaud, D. C. Horwell, R. A. Lewthwaite, J. Martinez and M. C. Pritchard, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1245.

152. Y. Wei and J. Konopelski, *Najing Ligong Daxue Xuebao*, 2000, **24**, 177.
153. M. Martin-Martinez, N. De la Figuera, M. Lattore, R. Herranz, M. T. Garcia-Lopez, E. Cenarruzabeitia, J. Del Rio and J. Gonzalez-Muniz, *J. Med. Chem.*, 2000, **43**, 3770.
154. P. Gouldson, P. Legoux, C. Carillon, B. Delpech, G. Le Fur, P. Ferrara and D. Shire, *Eur. J. Pharmacol.*, 2000, **400**, 185.
155. Y. Satoh, *J. Synth. Org. Chem. Japan*, 2000, **58**, 766.
156. M. A. Bednarek, S. D. Feighner, S. S. Pong, K. K. McKee, D. L. Hreniuk, M. V. Silva, V. A. Warren, A. D. Howard, L. H. Y. Van der Ploeg and J. V. Heck, *J. Med. Chem.*, 2000, **43**, 4370.
157. R. M. Scarborough and D. D. Gretler, *J. Med. Chem.*, 2000, **43**, 3453.
158. D. P. Chew and D. J. Moliterno, *J. Am. Coll. Cardiology*, 2000, **36**, 2028.
159. J. J. Ferguson and M. Zaqqa, *Drugs*, 1999, **58**, 965.
160. W. Wang, R. T. Borchardt and B. Wang, *Curr. Med. Chem.*, 2000, **7**, 437.
161. J. M. Smallheer, R. E. Olsen and R. R. Wexler, *Annu. Rep. Med. Chem.*, 2000, **35**, 103.
162. F. Q. Liu, R. M. Craft, S. A. Morris and R. C. Carroll, *Expert. Op. Invest. Drugs*, 2000, **9**, 2673.
163. T. M. Sielecki, J. Wityak, J. Liu, S. A. Mousa, M. Thoolen, R. R. Wexler and R. E. Olson, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 449.
164. F. Osterkamp, B. Ziemer, U. Koert, M. Wiesner, P. Raddatz and S. L. Goodman, *Chem.-Eur. J.*, 2000, **6**, 666.
165. S. Ono, Y. Inoue, T. Yoshida, A. Ashimori, K. Kosaka, T. Imada, C. Fukaya and N. Nakamura, *Chem. Pharm Bull.*, 1999, **47**, 1685.
166. A. Basso, N. Pegg, B. Evans and M. Bradley, *Eur. J. Org. Chem.*, 2000, 3887.
167. S. Ono, T. Yoshida, K. Maeda, K. Kosaka, Y. Inoue, T. Imada, C. Fukaya and N. Nakamura, *Chem. Pharm. Bull.*, 1999, **47**, 1694.
168. V. van Wyk, L. Leblond, P. D. Winocour, D. V. Devine and M. I. C. Gyongyossy-Issa, *Thromb. Res.*, 2000, **99**, 343.
169. Y. K. Kang and J. S. Jhon, *J. Pept. Res.*, 2000, **56**, 360.
170. M. Miyashita, M. Akamatsu, Y. Hayashi and T. Ueno, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 859.
171. S. Ono, Y. Inoue, T. Yoshida, K. Maeda, T. Imada, C. Fukaya and N. Nakamura, *Eur. J. Med. Chem.*, 2000, **35**, 577.
172. A. Peyman, J. F. Gourvest, T. R. Gadek and J. Knolle, *Angew. Chem. Int. Ed.* 2000, **39**, 2874.
173. A. Peyman, V. Wehner, J. Knolle, H. U. Stilz, G. Breipohl, K.-H. Scheunemann, D. Carniato, J.-M. Ruxer, J.-F. Gourvest, T. R. Gadek and S. Bodary, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 179.
174. E. Lahof, E. Planker, C. Mang, F. Burkhart, M. A. Dechantsreiter, R. Haubner, H.-J. Wester, M. Shwaiger, G. Hölzemann, S. L. Goodman and H. Kessler, *Angew. Chem. Int. Ed.*, 2000, **39**, 2761.
175. D. G. Batt, J. J. Petraitis, G. C. Houghton, D. P. Modi, G. A. Cain, M. H. Cain, M. H. Corjay, S. A. Mousa, P. J. Bouchard, M. S. Forsythe, P. P. Harlow, F. A. Barbera, S. M. Spitz, R. R. Wexler and P. K. Jadhav, *J. Med. Chem.*, 2000, **43**, 41.
176. J. S. Kerr, A. M. Slee and S. A. Mousa, *Expert Op. Invest. Drugs*, 2000, **9**, 1271.
177. G. D. Hartman and M. E. Duggan, *Expert Op. Invest. Drugs*, 2000, **9**, 1281.
178. S. C. Archibald, J. C. Head, J. M. Linsley, J. R. Porter, M. K. Robinson, A. Shock and G. J. Warrellow, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 993.
179. S. C. Archibald, J. C. Head, N. Gozzard, D. Howat, T. A. H. Parton, J. R. Porter, M.

K. Robinson, A. Shock, G. J. Warrellow and W. M. Abraham, *Bioorg. Med. Chem. Lett*., 2000, **10**, 997.
180. D. Cue, S. O. Southern, P. J. Southern, P. J. Prabhakar, W. Lorelli, J. M. Smallheer, S. A. Mousa and P. P. Cleary, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 2858.
181. J. Tilley, G. Kaplan, N. Fotouhi, B. Wolitzki and K. Rowan, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1163.
182. N. Fotouhi, P. Joshi, J. Tilley, K. Owen, V. Schwinge and B. Wolitzky, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1167.
183. N. Fotouhi, P. Joshi, D. Fry, C. Cook, J. W. Tilley, G. Kaplan, A. Hanglow, K. Rowan, V. Schwinge and B. Wolitzky, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1171.
184. X. M. Gao, Y. C. Tang and Y. H. Ye, *Prog. Biochem. Biophys*., 2000, **27**, 500.
185. T. Reismann, A. V. Schally, P. Bouchard, H. Riethmuller and J. Engel, *Human Rep. Update*, 2000, **6**, 322.
186. D. Yahalom, S. Rahimipour, Y. Kock, N. Ben-Aroya and M. Fridkin, *J. Med. Chem*., 2000, **43**, 2831.
187. J. Hlaváèek, B. Bennettová, R. Tykva, J. Velek, V. Kašièka and T. Barth, *Lett. Pept. Sci*., 2000, **7**, 85.
188. J. E. Rivier, R. S. Struthers, J. Porter, S. L. Lahrichi, G. Jiang, L. A. Cervini, M. Ibea, D. A. Kirby, S. C. Koerber and C. L. Rivier, *J. Med. Chem*., 2000, **43**, 784.
189. J. E. Rivier, J. Porter, L. A. Cervini, S. L. Lahrichi, D. A. Kirby, R. S. Struthers, S. C. Koerber and C. L. Rivier, *J. Med. Chem*., 2000, **43**, 797.
190. J. E. Rivier, G. Jiang, R. S. Struthers, S. C. Koerber, J. Porter, L. A. Cervini, D. A. Kirby, A. G. Craig and C. L. Rivier, *J. Med. Chem*., 2000, **43**, 807.
191. S. C. Koerber, J. Rizo, R. S. Struthers and J. E. Rivier, *J. Med. Chem*., 2000, **43**, 819.
192. M. R. Lerner, *Cutaneous Neuroimmun*: *Proopiomelanocortin Syst*., 1999, **885**, 153.
193. J. M. Lipton, H. Zhao, T. Ichiyama, G. S. Barsh and A. Catania, *Cutaneous Neuroimmun*: *Proopiomelanocortin Syst*., 1999, **885**, 173.
194. A. Catania, R. Delgado, L. Airaghi, M. Cutuli, L. Garofalo, A. Carlin, M. T. Demitri and J. M. Lipton, *Cutaneous Neuroimmun*: *Proopiomelanocortin Syst*., 1999, **885,** 183.
195. A. J. Thody, *Cutaneous Neuroimmun*: *Proopiomelanocortin Syst*., 1999, **885**, 217.
196. M. C. Cremer, M. S. Sanchez and M. E. Celis, *Peptides*, 2000, **21**, 803.
197. M. A. Bednarek, T. MacNeil, R. N. Kalyani, R. Tang, L. H. T. Van der Ploeg and D. H. Weinberg, *Biochem. Biophys. Res. Commun*., 2000, **272**, 23.
198. M. Nakagawa, K. Chiba-Kamoshida, K. Udaka and H. Nakanishi, *Biochem. Biophys. Res. Commun*., 2000, **278**, 609.
199. T. L. Chapman, A. P. Heikema, A. P. West and P. J. Bjorkman, *Immunity*, 2000, **13**, 727.
200. G. Guichard, A. Zerbib, F. A. LeGal, J. Hoebke, F. Connan, J. Choppin, J. P. Briand and J. G. Guillet, *J. Med. Chem*., 2000, **43**, 3803.
201. A. R. Khan, B. M. Baker, P. Ghosh, W. E. Biddison and D. C. Wiley, *J. Immun*., 2000, **164**, 6398.
202. A. L. Zarling, S. B. Ficarro, J. A. Shabonawitz, D. F. Hunt and V. H. Engelliard, *FASEB J*. 2000, **14**, A1192.
203. O. Michielin, I. Luescher and M. Karplus, *J. Mol Biol*., 2000, **300**, 1205.
204. O. Schueler-Furman, Y. Altuvia, A. Sette and H. Margalit, *Protein Sci*., 2000, **9**, 1838.
205. L. Stoltze, M. Schirle, G. Schwarz, C. Schroter, M. W. Thompson, L. B. Hersh, H. Kalbacher, S. Stevanovic, H. G. Rammensee and H. Schild, *Nature Immun*., 2000, **1**, 413.

206. L. Ducry, S. Reinelt, P. Seiler, F. Diedrich, D. R. Bolin, R. M. Campbell and G. L. Olson, *Helv. Chim. Acta*, 1999, **82**, 2432.
207. E. Wellner, T. Gustafsson, J. Backlund, R. Holmdahl and J. Kihlberg, *ChemBiochem*, 2000, **1**, 272.
208. D. R. Bolin, A. L. Swain, R. Sarabu, S. J. Berthel, P. Gillespie, H. J. S. Huby, R. Makofske, L. Orzechowski, A. Perrotta, K. Toth, J. P. Copper, N. Jiang, F. Falcioni, R. Campbell, D. Cox, D. Gaizband, C. J. Belunis, D. Vidovic, K. Ito, R. Crowther, U. Kammlott, X. L. Zhang, R. Palermo, D. Weber, J. Guenot, Z. Nagy and G. L. Olson, *J. Med. Chem.*, 2000, **43**, 2135.
209. R. E. Humphreys, S. Adams, G. Koldzic, B. Nedelescu, E. von Hofe, and M. Xu, *Vaccine*, 2000, **18**, 2693.
210. M. Hakansson, K. Petersson, H. Nilsson, G. Forsberg, P. Bjork, P. Antonsson and L. A. Svensson, *J. Mol. Biol.*, 2000, **302**, 527.
211. D. Muno, E. Kominami and T. Mizochi, *FEBS Lett.*, 2000, **478**, 178.
212. D. M. Fernandes, L. Vidard and K. L. Rock, *Eur. J. Immun.* 2000, **30**, 2333.
213. G. Bonomi, F. Moschella, M. N. Ombra, G. Del Pozzo, C. Granier, P. De Berardinis and J. Guardiola, *Human Immun.*, 2000, **61**, 753.
214. S. J. Turley, K. Inaba, W. S. Garrett, M. Ebersold, J. Unternaehrer, R. M. Steinman and I. Mellman, *Science*, 2000, **288**, 522.
215. G. H. Tishkoff and L. T. Hunt, *Thromb. Res.*, 2000, **98**, 343.
216. C. Cabrele and A. G. Beck-Sickinger, *J. Pept. Sci.*, 2000, **6**, 97.
217. C. P. Eckard and A. G. Beck-Sickinger, *Curr. Med. Chem.*, 2000, **7**, 897.
218. A. W. Stamford and E. M. Parker, *Ann. Rep. Med. Chem.*, 1999, **34**, 31.
219. P. K. Ganguly, *J. Health Sci.*, 2000, **46**, 430.
220. A. W. Lloyd, *Drug Discovery Today*, 2000, **5**, 480.
221. Y. Takebayashi, H. Koga, J. Togami, A. Inui, H. Kurihara, T. Furuya, A. Tanaka and K. Murase, *Chem. Pharm. Bull.*, 2000, **48**, 1925.
222. K. Racaityte, E. S. M. Lutz, K. K. Unger, D. Lubda and K. S. Boos, *J. Chromatog. A*, 2000, **890**, 135.
223. E. Salaneck, S. K. S. Holmberg, M. T. Berglund, T. Boswell and D. Larhammar, *FEBS Letters*, 2000, **484**, 229.
224. J. M. Cerd-Reverter, G. Martinez-Rodriguez, S. Zanuy, M. Carrillo and D. Larhammar, *Regulatory Pept.*, 2000, **95**, 25.
225. J. M. Cerd-Reverter and D. Larhammar, *Biochem. Cell Biol.*, 2000, **78**, 371.
226. E. M. Parker, A. Balasubramaniam, M. Guzzi, D. E. Mullins, B. G. Salisbury, S. Sheriff, M. B. Witten and J. J. Hwa, *Peptides*, 2000, **21**, 393.
227. C. M. Brown, D. V. Coscina and P. J. Fletcher, *Peptides*, 2000, **21**, 1279.
228. V. J. Hruby and R. S. Agnes, *Biopolymers*, 1999, **51**, 391.
229. C. Dooley and R. A. Houghten, *Biopolymers*, 1999, **51**, 379.
230. E. Y. K. Huang, J. Y. Li, P. P. C. Tan, C. H. Wong and J. C. Chen, *Peptides*, 2000, **21**, 205.
231. P. Panula, E. Kalso, M. Nieminen, A. Brandt and A. Pertovaara, *Brain Res.*, 1999, **848**, 67.
232. L. Prokai, A. Zharikova, A. Juhasz and K. Prokai-Tatrai, *Brain Res.*, 1999, **848**, P218.
233. M. J. M. A. Nijsen, G. J. W. de Ruiter, C. M. Kasbergen, P. Hoogerhout and D. J. de Wildt, *Br. J. Pharmacology*, 2000, **131**, 1468.
234. E. J. Bilsky, X. H. Qian, V. J. Hruby and F. Porreca, *J. Pharmacol and Exp. Therap.*, 2000, **293**, 151.
235. S. F. Martin, M. P. Dwyer, B. Hartmann and K. S. Knight, *J. Org. Chem.*, 2000, **65**, 1305.

236. Z. S. Arnold and P. W. Schiller, *J. Pept. Sci.*, 2000, **6**, 280.
237. D. Y. Maeda, J. E. Ishmael, T. F. Murray and J. V. Aldrich, *J. Med. Chem.*, 2000, **43**, 3941.
238. J. P. Malkinson, R. A. Falconer and I. Toth, *J. Org. Chem.*, 2000, **65**, 5249.
239. T. K. Chakraborty, S. Ghosh, S. Jayaprakash, J. A. R. P. Sharma, V. Ravikanth, P. V. Diwan, R. Nagaraj and A. C. Kunwar, *J. Org. Chem.*, 2000, **65**, 6441.
240. R. D. Egleton, S. A. Mitchell, J. D. Huber, J. Janders, D. Stropova, R. Polt, H. I. Yamamura, V. J. Hruby and T. P. Davis, *Brain Res.*, 2000, **881**, 37.
241. C. T. Kriss, B. S. Lou, L. Z. Szabo, S. A. Mitchell, V. J. Hruby and R. Polt, *Tetrahedron-Asymmetry*, 2000, **11**, 9.
242. E. J. Bilsky, R. D. Egleton, S. A. Mitchell, M. M. Palian, P. Davis, J. D. Huber, H. Jones, H. I. Yamamura, J. Janders, T. P. Davis, F. Porreca, V. J. Hruby and R. Polt, *J. Med. Chem.*, 2000, **43**, 2586.
243. E. Vass, M. Hollosi, M. Kveder, B. Kojic-Prodic, M. Cudic and S. Horvat, *Spectrochim. Acta Pt. A Mol. Biomol. Spectr.*, 2000, **56**, 2479.
244. K. W. Kim, R. S. Woo, C. J. Kim, Y. P. Cheong, J. K. Kim, J. Kwun and K. P. Cho, *Life Sci.*, 2000, **67**, 61.
245. B. Bozo, J. Farkas, G. Toth, M. Wollemann, M. Szucs and S. Benhye, *Life Sci.*, 2000, **66**, 1241.
246. T. P. Su, *J. Biomed. Sci.*, 2000, **7**, 195.
247. H. X. Chen, F. Noble, A. Mothe, H. Meudal, P. Coric, S. Danascimento, B. P. Roques, P. George and M. C. Fournie-Zaluski, *J. Med. Chem.*, 2000, **43**, 1398.
248. D. M. Desiderio, U. Wirth, J. L. Lovelace, G. Fridland, E. S. Umstot, T. M. D. Nguyen, P. W. Schiller, H. S. Szeto and J. F. Clapp. *J. Mass. Spec.*, 2000, **35**, 725.
249. L. M. Sun and J. A. Gardella, *Pharm. Res.*, 2000, **17**, 859.
250. M. D. Shenderovich, S. B. Liao, X. H. Qian and V. J. Hruby, *Biopolymers*, 2000, **53**, 565.
251. H. I. Mosberg, *Biopolymers*, 1999, **51**, 426.
252. M. J. Ji and P. C. Yang, *Acta Physico-Chimica Sinica*, 2000, **16**, 596.
253. M. Kinoshita, Y. Okamoto and F. Hirata, *J. Am. Chem. Soc.*, 2000, **122**, 2773.
254. S. Antolic, M. Teichert, G. Sheldrick, B. Kojic-Prodic, M. Cudic and S. Horvat, *Acta Cryst. Sect. B.-Struct. Sci.*, 1999, **55**, 975.
255. P. W. Schiller, T. M. D. Nguyen, I. Berezowska, S. Dupuis, G. Weltrowska, N. N. Chung and C. Lemieuz, *Eur. J. Med. Chem.*, 2000, **35**, 895.
256. I. J. McFadyen, K. Sobczyk-Kojiro, M. J. Schaefer, J. C. Ho, J. R. Omnaas, H. I. Mosberg and J. R. Traynor, *J. Pharmac. Exp. Therapeutics*, 2000, **295**, 960.
257. Y. Okada, A. Fukumizu, M. Takahashi, Y. Shimizu, Y. Tsuda, T. Yokoi, S. D. Bryant and L. H. Lazarus, *Biochem. Biophys. Res. Commun.*, 2000, **276**, 7.
258. A. Olma and D. Tourwé, *Lett. Pept. Sci.*, 2000, **7**, 93.
259. S. Sakurada, S. Takeda, T. Sato, T. Hayashi, M. Yuki, M. Kutuwa, K. Tan-No, C. Sakurada, K. Kisara and T. Sukurada, *Eur. J. Pharmac.*, 2000, **395**, 107.
260. I. J. McFayaden, J. C. Ho, H. I. Mosberg and J. R. Traynor, *J. Pept. Res.*, 2000, **55**, 255.
261. M. P. Allen, J. F. Blake, D. K. Bryce, M. E. Haggan, S. Liras, S. McLean and B. E. Segelstein, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 523.
262. P. W. Schiller, G. Weltrowska, I. Berezowska, T. M. D. Nguyen, B. C. Wilkes, C. Lemieux and N. N. Chung, *Biopolymers*, 1999, **51**, 411.
263. R. Guerrini, G. Calo, R. Bigoni, A. Rizzi, K. Varani, G. Toth, S. Gessi, E. Hashiba, Y. Hashimoto, D. C. Lambert, P. A. Borea, R. Tomatis, S. Salvadori and D. Regoli, *J. Med. Chem.*, 2000, **43**, 2805.

264. G. Calo, R. Guerrini, R. Bigoni, A. Rizzi, G. Marzola, H. Okawa, C. Bianchi, D. G. Lambert, S. Salvadori and D. Regoli, *Br. J. Pharmacol*., 2000, **129**, 1183.
265. K. Okada, T. Sujaku, Y. Chuman, R. Nakashima, T. Nose, T. Costa, Y. Yamada, M. Yokoyama, A. Nagahisa and Y. Shimohigashi, *Biochem. Biophys. Res. Commun*., 2000, **278**, 493.
266. Y. Jinsmaaa, M. Takahashi, H. Fukunaga and M. Yoshikawa, *Life Sci*., 2000, **67**, 3095.
267. R. Guerrini, G. Calo, A. Rizzi, R. Bigoni, D. Rizzi, D. Regoli and S. Salvadori, *Peptides*, 2000, **21**, 923.
268. G. Calo, R. Guerrini, A. Rizzi, S. Salvadori and D. Regoli, *Br. J. Pharmacol*., 2000, **129**, 1261.
269. C. T. Dooley and R. A. Houghten, *Peptides*, 2000, **21**, 949.
270. J. C. Meunier, L. Mouledous and C. M. Topham, *Peptides*, 2000, **21**, 893.
271. L. Mouledos, C. M. Topham, H. Mazarguil and J. C. Meunier, *J. Biol. Chem*., 2000, **275**, 29268.
272. H. Shinkai, T. Ito, T. Iida, Y. Kitao, H. Yamada and I. Uchida, *J. Med. Chem*., 2000, **43**, 4667.
273. S. Ozaki, H. Kawamoto, Y. Itoh, M. Miyaji, T. Azuma, D. Ichikawa, H. Nambu, T. Iguchi, Y. Iwasawa and H. Ohta, *Eur. J. Pharmac*., 2000, **402**, 45.
274. C. Thomsen and R. Hohlweg, *Br. J. Pharmac*., 2000, **131**, 903.
275. J. Wichmann, G. Adam, S. Rover, M. Hennig, M. Scalone, A. M. Cesura, F. M. Dautzenberg and F. Jenck, *Eur. J. Med. Chem*., 2000, **35**, 839.
276. S. Rover, G. Adam, A. M. Cesura, G. Galley, F. Jenck, F. J. Monsma, J. Wichmann and F. M. Dautzenberg, *J. Med. Chem*., 2000, **43**, 1329.
277. E. Okuda-Ashitaka and S. Ito, *Peptides*, 2000, **21**, 1101.
278. O. Crescenzi, R. Guerrini, D. Picone, S. Salvadori, T. Tancredi and P. A. Temussi, *Biopolymers*, 2000, **53**, 257.
279. T. Honda, N. Shirasu, Y. Chuman *et. al*., *Bull. Chem. Soc. Jap*., 2000, **73**, 2549.
280. D. L. Heyl, M. D. Sanvordekar, G. Dogruyol, M. D. Salamoun, D. W. Rodgers, K. Renganathan, C. Mousigian and S. E. Schullery, *Prot. Pept. Lett*., 1999, **6**, 359.
281. Z. Darula, K. E. Kover, K. Monory, A. Borsodi, E. Mako, A. Ronai, D. Tourwé, A. Peter and G. Toth, *J. Med. Chem*., 2000, **43**, 1359.
282. I. Tooyama, H. Abe, T. G. Renda and H. Kimura, *Peptides*, 2000, **21**, 1649.
283. G. Schletingen, L. Zhang, A. Maycock, R. N. DeHaven, J. D. Dawbert, J. Cassel, N. N. Chung, P. W. Schiller and M. Goodman, *J. Med. Chem*., 2000, **43**, 2698.
284. G. Ronsisville, L. Pasquinucci, V. Pittala, A. Marazzo, O. Prezzavento, R. Di Torro, B. Falcucci and S. Spampinato, *J. Med. Chem*., 2000, **43**, 2992.
285. L. Leelasvatanakiy and J. V. Aldrich, *J. Pept. Res*., 2000, **56**, 80.
286. D. M. Ferguson, S. Kramer, T. G. Metzer, P. Y. Law and P. S. Portoghese, *J. Med. Chem*., 2000, **43**, 1251.
287. X. H. Wan, X. Q. Huang, D. H. Zhou, H. L. Jiang, K. X. Chen and Z. Q. Chi, *Acta Pharmacol. Sinica*, 2000, 21, 701; X. H. Wan, X. Q. Huang, D. H. Zhou, H. L. Jiang, K. X. Chen, Z. Q. Chi and Y. P. Feng, *Prot. Pept. Lett*., 2000, **7**, 403.
288. R. Sankararamakrishnan and H. Weinstein, *Biophys. J*., 2000, **79**, 2331; *ibid*., 2000, **78**, 1896P.
289. A. V. Schally, A. M. Comaru-Schally, A. Plonowski, A. Nagy, G. Halmos and Z. Rekasi, *Prostate*, 2000, **45**, 158.
290. N. R. A. Beeley, *Drug Discovery Today*, 2000, **5**, 354.
291. A. J. Souers, A. Rosenquist, E. M. Jarvie, M. Ladlow, W. Fenuik and J. A. Ellman, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2731.

292. D. J. Suich, S. A. Mousa, G. Singh, G. Liapakis, T. Reisine and W. F. Grado, *Bioorg. Med. Chem.*, 2000, **8**, 2229.
293. D. Besser, B. Muller, P. Kleinwachter, G. Greiner, L. Seyfarth, T. Steinmetzer, O. Arad and S. Reissmann, *J. Prakt. Chem.*, 2000, **342**, 537.
294. G. P. Cartoni, F. Coccioli, R. Jasionwska and M. Masci, *J. Pharm. Biochem. Anal.*, 2000, **23**, 143.
295. J. Vanden Broeck, H. Torfs, J. Poels, W. VanPoyer, E. Swinnen, K. Ferket and A. De Loof, *Neuropeptides: Struct. Funct. Biol. Behaviour*, 1999, **897**, 374.
296. V. Leroy, P. Mauser, Z. L. Gao and N. P. Peet, *Expert. Opin. Investigational Drugs*, 2000, **9**, 735.
297. C. Swain and N. M. J. Rupniak, *Annu. Rep. Med. Chem.*, 1999, **34**, 51.
298. M. S. Kramer, *Neuropeptides*, 2000, **34**, 255.
299. D. G. Snijdelaar, R. Dirksen, R. Slappendel and B. J. P. Crul, *Eur. J. Pain-London*, 2000, **4**, 121.
300. R. Hill, *Trends Pharmacol. Sci.*, 2000, **21**, 244.
301. J. Liu, D. J. Underwood, M. A. Cascieri, S. P. Rohrer, L.-D. Cantin, G. Chicchi, A. B. Smith and R. Hirschmann, *J. Med. Chem.*, 2000, **43**, 3827.
302. Y. S. Tong, Y. M. Fobian, M. Y. Wu, N. D. Boyd and K. D. Moeller, *J. Org. Chem.*, 2000, **65**, 2484.
303. S. E. Foran, D. B. Carr, A. W. Lipkowski, I. Maszczynska, J. E. Marchand, A. Misicka, M. Beinborn, A. S. Kopin and R. M. Kream, *Proc. Natl Acad. Sci. USA*, 2000, **97**, 7621.
304. J. Sinnett-Smith, C. Santiskulvong, J. Duque and E. Rozengurt, *J. Biol. Chem.*, 2000, **275**, 30644.
305. X. J. Wu, S. Toppet, F. Compernolle and G. J. Hoornaert, *Tetrahedron*, 2000, **56**, 6279.
306. R. T. Lewis, T. Ladduwahetty, K. J. Merchant, L. E. Keown, L. Hitzel, H, Verrier, G. I. Stevenson and A. M. MacLeod, *J. Org. Chem.*, 2000, **65**, 2615.
307. X.-F. Qi, B. S. Zharov and V. S. Ananthanarayanan, *J. Pept. Sci.*, 2000, **6**, 57.
308. A. A. Bremer, S. E. Leeman and N. D. Boyd, *FEBS Lett.* 2000, **486**, 43.
309. S. Auge, B. Bersch, M. Tropis and A. Milon, *Biopolymers*, 2000, **54**, 297.
310. S. A. Perrine, T. L. Whitehead, R. P. Hicks, J. L. Szarek, J. E. Krause and M. A. Simmons, *J. Med. Chem.*, 2000, **43**, 1741.
311. T. C. Wong and T. W. Wymore, *Biophys. J.*, 2000, **78**, 1913P.
312. A. Kamel, C. Prakash, C. Saverino, B. Obach and H. Fouda, *J. Chromatog. B*, 2000, **748**, 179.
313. M. Gerspacher, A. von Sprecher, R. Mah, G. P. Anderson, C. Bertrand, N. Subramanian, K. Hauser and H. A. Ball, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1467.
314. H. M. Sarau, D. E. Griswold, B. Bush, W. Potts, P. Sandhu, D. Lundberg, J. J. Foley, D. B. Schmidt, E. F. Webb, L. D. Martin, J. J. Legos, R. G. Whitmore, F. C. Barone, A. D. Medhurst, M. A. Luttmann, G. A. M. Giardina and D. W. P. Hay, *J. Pharmacol. Exp. Therapeutics*, 2000, **295**, 373.
315. A. J. Carnell, M. L. E. Hernandez, A. Pettman and J. F. Bickley, *Tetrahedron Lett.*, 2000, **41**, 6929.
316. T. Nishi, K. Ishibashi, T. Takemoto, K. Nakajima, T. Fukazawa, Y. Iio, K. Itoh, O. Mukaiyama and T. Yamaguchi, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1665.
317. J. J. Hale, S. G. Mills, M. MacCoss, C. P. Dorn, P. E. Finke, R. J. Budhu, R. A. Reamer, S. E. W. Huskey, D. Luffer-Atlas, B. J. Dean, E. M. McGowan, W. P. Feeney, S. H. L. Chiu, M. A. Cascieri, C. G. Chicchi, M. M. Kurtz, S. Sadowski, E. Ber, F. D. Tattersall, N. M. J. Rupniak, A. R. Williams, W. Rycroft, R. Hargreaves,

J. M. Metzger and D. E. MacIntyre, *J. Med. Chem.*, 2000, **43**, 1234.
318. A. Vedani, H. Briem, M. Dobler, H. Dollinger and D. McMasters, *J. Med. Chem.*, 2000, **43**, 4416.
319. S. Valenza, F. M. Cordero, A. Brandi, A. Guidi, M. Altamura, A. Giolitti, F. Giuntini, F. Pasqui, A. R. Renzetti and C. A. Maggi, *J. Org. Chem.*, 2000, **65**, 4003.
320. S. Rodziewicz-Motowidlo, A. Legowska, X.-F. Qi, C. Czaplewski, A. Liwo, P. Sowinski, M. Mozga, J. Olczak, J. Zabrocki and K. Rolka, *J. Pept. Res.*, 2000, **56**, 132.
321. D. Giannotti, E. Perrotta, C. Di Bugno, R. Nannicini, N. J. S. Harmat, A. Giolitti, R. Patacchini, A. R. Renzetti, L. Rotondaro, S. Guiliani, M. Altamura and C. A. Maggi, *J. Med. Chem.*, 2000, **43**, 4041.
322. S. Greenfeder, B. Cheewatrakoolpong, M. Billah, R. W. Egan, E. Keene, N. J. Murgolo and J. C. Anthes, *Bioorg. Med. Chem.*, 1999, **7**, 2867.
323. S. Y. Shin, J. K. Bang, J. S. Park and S. W. Kang, *Bull. Korean Chem. Soc.*, 2000, **21**, 932.
324. C. A. Lewis, A. Steward, N. Subramanian and J. R. Fozard, *Br. J. Pharmacol.*, 2000, **131**, 118P.
325. D. Schutze, B. Boss and J. Schmid, *J. Chromatogr. B*, 2000, **748**, 55.
326. Z. Procházka, M. Zertová and J. Slaninová, *Lett. Pept. Sci.*, 2000, **7**, 179.
327. L. Belec, J. Slavinova and W. D. Lubell, *J. Med. Chem.*, 2000, **43**, 1448.
328. J. F. Novak, M. B. Judkins, M. I. Chernin, P. Cassoni, G. Bussolati, J. A. Nitche and S. K. Nishimoto, *Cancer Res.*, 2000, **60**, 3470; J. F. Novak, *ibid.*, 2000, **60**, 4663.
329. H. Wei, X. M. Luo, Y. B. Wu, Y. Yao, Z. J. Guo and L. G. Zhu, *J. Chem. Soc. Dalton Trans.*, 2000, 4196.
330. A. Rachkov and N. Minoura, *J. Chromatogr. A*, 2000, **889**, 111.
331. M. Birnbaumer, *Trends in Endocrin. Metabol.*, 2000, **11**, 406.
332. M. Sobocinska, E. Lempicka, E. Konieczna, I. Derdowska, B. Lammek, S. Melhem, W. Kozik, J. Janecka, M. Janecki and H. I. Trzeciak, *J. Pharm. Pharmac.*, 2000, **52**, 1105.
333. M. Zaoral, I. Bláha, M. Budešinsky, A. Machová and J. Slaninová, *J. Pept. Sci.*, 2000, **6**, 123.
334. K. Kondo, H. Ogawa, T. Shinohara, M. Kurimara, Y. Tamada, K. Kan, H. Yamashita, S. Nakamura, T. Hirano, Y. Yamamura, T. Mori, M. Tominaga and A. Atai, *J. Med. Chem.*, 2000, **43**, 4388.
335. M. A. Ashwell, J. F. Bagli, T. J. Caggiano, P. S. Chen, A. J. Molinari, C. Palka, C. H. Park, J. F. Rogers, M. Sherman, E. J. Trybulski and D. K. Williams, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 783.
336. J. D. Albright, E. G. Delos Santos, J. P. Dusza, P. S. Chan, J. Coupet, X. Ru and H. Mazandarani, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 695.
337. A. Matsuhisa, N. Taniguchi, H. Koshio, T. Yatsu and A. Tanaka, *Chem. Pharm. Bull.*, 2000, **48**, 21.
338. Y. Shimada, N. Taniguchi, A. Matsuhisa, K. Sakamoto, T. Yatsu and A. Tanaka, *Chem. Pharm. Bull.*, 2000, **48**, 1644.
339. J. Matsubara, K. Kitano, K. Otsubo, Y. Kawano, T. Ohtani, M. Bando, M. Kido, M. Uchida and F. Tabusa, *Tetrahedron*, 2000, **56**, 4667.
340. N. Cotte, M. N. Balestre, A. Aumelas, E. Mahe, S. Phalipou, D. Morin, M. Hibert, M. Manning, T. Durroux, C. Barberis and B. Mouillac, *Eur. J. Biochem.*, 2000, **267**, 4253.
341. M. Thibonnier, P. Coles, D. M. Conarty, C. L. Plesnicher and M. Shoham, *J. Pharmac. Exp. Therapeutics*, 2000, **294**, 195.

342. S. R. Hawtin, V. J. Wesley, R. A. Parslow, S. Patel and M. Wheatley, *Biochemistry*, 2000, **39**, 13524.
343. R. Slusarz, R. Kazmierkiewicz and B. Lammek, *J. Pept. Res.*, 2000, **56**, 352.
344. M. Iwadate, E. Nagao, M. P. Williamson, M. Ueki and T. Asukara, *Eur. J. Biochem.*, 2000, **267**, 4504.
345. H. Zahn, *J. Pept. Sci.*, 2000, **6**, 1.
346. D. Fiertek, M. Gromowska, A. S. Andersen, P. H. Hansen, T. Majewski and J. Izdebski, *J. Pept. Sci.*, 2000, **6**, 413.
347. M. B. Norrevang, F. Abildgaard, S. M. Kristensen and J. J. Led, *Asian Chem. Lett.*, 2000, **4**, 159.
348. M. Schlein, S. Havelund, C. Kristensen, M. F. Dunn and N. C. Kaarsholm, *J. Mol. Biol.*, 2000, **303**, 161.
349. A. Oliva, J. Farina and M. Llabres, *J. Chromatogr. B.* 2000, **749**, 25.
350. P. Tito, E. J. Nettleton and C. V. Robinson, *J. Mol. Biol.*, 2000, **303**, 267.
351. R. Horuk and H. P. Ng, *Med. Res. Rev.*, 2000, **20**, 155.
352. I. Clark-Lewis, *Methods. Mol. Biol.*, 2000, **138**, 47.
353. J. R. White, J. M. Lee, K. Dede, C. S. Imburgia, A. J. Jurewicz, G. Chan, J. A. Fornwald, D. Dhanak, L. T. Christmann, M. G. Darcy, K. L. Widdowson, J. J. Foley, D. B. Schmidt and H. M. Sarau, *J. Biol. Chem.*, 2000, **275,** 36626.
354. T. Mirzadegan, F. Diehl, B. Ebi, S. Bhakta, I. Polsky, D. McCarley, M. Mulkins, G. S. Weatherhead, J. M. Lapierre, J. Dankwardt, D. Morgans, R. Wilhelm and K. Jarnagin, *J. Biol. Chem.*, 2000, **275**, 25562.
355. K. Konishi, K. Ikeda, K. Achiwa, H. Hoshino and K. Tanaka, *Chem. Pharm. Bull.*, 2000, **48**, 308.
356. H. R. Luttichau, J. Stine, T. P. Boesen, A. H. Johnsen, D. Chantry, J. Gerstoft and T. W. Schwartz, *J. Expt. Med.*, 2000, **191**, 171.
357. P. D. Bailey, C. A. R. Boyd, J. R. Bronk, I. D. Collier, D. Meredith, K. M. Morgan and C. S. Temple, *Angew. Chem. Int. Ed.*, 2000, **39**, 506.
358. Y.-C. Chen, I. Bab, N. Mansur, A. Muhlrad, A. Shteyer, N. Namdar-Attar, H. Gavish, M. Vidson and M. Chorev, *J. Pept. Res.*, 2000, **56**, 147.
359. N. Beglova, S. Maliartchouk, I. Ekiel, M. C. Zaccaro, H. U. Saragovi and K. Gehring, *J. Med. Chem.*, 2000, **43**, 3530.
360. P. J. Connolly, S. K. Wetter, W. V. Murray, D. L. Johnson, F. J. McMahon, F. X. Ferzell, J. Tullai and L. K. Jolliffe, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1995.
361. B. P. England, P. Balasubramanian, I. Uings, S. Bethell, M.-J. Chen, P. Schatz, Q. Yin, Y.-F. Chen, E. A. Whitehorn, A. Tsavaler, C. L. Martens, R. W. Barrett and M. McKinnon, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6862.
362. B.-S. Yun, I.-D. Yoo, Y. H. Kim, Y.-S. Kim, S. J. Lee, K.-S. Kim and W. H. Yeo, *Tetrahedron Lett.*, 2000, **41**, 1429.
363. S. K. Sharma, M. Tandon and J. W. Lown, *Eur. J. Org. Chem.*, 2000, 2095.
364. P. Cozzi, I. Beria, M. Caldarelli, L. Capalongo, C. Geroni and N. Mongelli, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1269; P. Cozzi, I. Beria, M. Caldarelli, C. Geroni, N. Mongelli and G. Pennella, *ibid.*, 2000, **10**, 1273.
365. P. G. Baraldi, R. Romagnoli, A. Duatti, C. Bolzati, A. Piffanelli, N. Bianco, C. Mischiati and R. Gambari, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1397.
366. F. Sasse, H. Steinmetz, J. Heil, *et. al.*, *J. Antibiotics*, 2000, **53**, 879.
367. S. Kokubo, K. Suenaga, C. Shinohara, T. Tsuji and D. Uemura, *Tetrahedron*, 2000, **56**, 6435.
368. F. Yokokawa, K. Izumi, J. Omata and T. Shioiri, *Tetrahedron*, 2000, **56**, 3027.
369. C. J. Leitheiser, M. J. Rishel, X. Wu and S. M. Hecht, *Org. Lett.*, 2000, **2**, 3397.

370. A. Giordano, C. D. Monica, F. Landi, A. Spinella and G. Sodano, *Tetrahedron Lett*., 2000, **41**, 3979.
371. J. I. Kourie and A. A. Shorthouse, *Am. J. Physiol.-Cell Physiol*., 2000, **278**, C1063.
372. P. A. Raj, K. J. Antonyraj and T. Karunakaran, *Biochem. J*., 2000, **347**, 633.
373. C. Haubrich, V. Frielingsdorf, S. Herzig, H. Schroder, R. Schwarting, V. Sturm and J. Voges, *Brain Res*., 2000, **855**, 225.
374. Z.-X. Guo, A. N. Cammidge and D. C. Horwell, *Tetrahedron*, 2000, **56**, 5169.
375. E. Drakapoulou, K. Uray, G. Mezo, M. R. Price, C. Vita and F. Hudecz, *J. Pept. Sci*., 2000, **6**, 175.
376. P. Gilligan, D. W. Robertson and R. Zaczek, *J. Med. Chem*., 2000, **43**, 1641.
377. Y. Zhang, W. Wu, M. Wang and T. Wu, *Zhongugguo Yaowu*, *Huaxue Zazhi*, 2000, **10**, 124.
378. D. Trivedi, Y. Lin, J. M. Ahn, M. Siegel, N. N. Mollova, K. H. Schram and V. J. Hruby, *J. Med. Chem*., 2000, **43**, 1714.
379. M. N. Mathieu, J. D. Wade, Y.-Y.Tan, R. J. Summers and G. W. Tregear, *J. Pept. Sci*., 2000, **6**, 235.
380. M. Kossravi and R. T. Borchardt, *Pharm. Res*., 2000, **17**, 851.
381. S. E. Blondelle, E. Crooks, R. Aligue, N. Agell, O. Bachs, V. Esteve, R. Tejero, B. Celda, M. T. Paster and E. Perez-Paya, *J. Pept. Res*., 2000, **55**, 148.
382. T. Ohyama, H. Oku, A. Hiroki *et. al*., *Biopolymers*, 2000, **54**, 375.
383. V. Sanz-Nebot, F. Benavente and J. Barbosa, *J. Chromatogr. A*, 2000, **870**, 315.
384. A. Ray and B. Norden, *FASEB J*., 2000, **14**, 1041.
385. D. Leung, G. Abbenante and D. P. Fairlie, *J. Med. Chem*., 2000, **43**, 305.
386. J. Deadman, *J. Pept. Sci*., 2000, **6**, 421.
387. A. J. Rivett and R. C. Gardner, *J. Pept. Sci*., 2000, **6**, 478.
388. C. N. Shrimpton and I. A. Smith, *J. Pept. Sci*., 2000, **6**, 251.
389. E. S. H. El Ashry, N. Rashed and A. H. S. Shobier, *Pharmazie*, 2000, **55**, 403.
390. G. S. Hamilton and C. Thomas, *Adv. Med. Chem*., 2000, **5**, 1.
391. H. Takahashi, M. Komoda, H. Kakuta and Y. Hashimoto, *J. Pharm. Soc. Jap*., 2000, **120**, 909.
392. J. Grembecka, W. A. Sokalski and P. Kafarski, *J. Comp.-Aided Mol. Design*, 2000, **14**, 531.
393. I. O.Donkor, *Curr. Med. Chem*., 2000, **7**, 1171.
394. A. Stracher, *Ototoxicity*: *Basic Sci. and Clin. Appl*., 1999, **884**, 52.
395. W. Lubisch, H. P. Hofmann, H. J. Treiber and A. Moller, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2187.
396. R. Tipathy, M. A. Ator and J. P. Mallamo, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2315.
397. I. O. Donkor, X. Z. Zheng, J. Han and D. D. Miller, *Chirality*, 2000, **12**, 551.
398. K. C. Chou, A. G. Tomasselli and R. L. Heinrikson, *FEBS Lett*., 2000, **470**, 249.
399. D. Lee, S. A. Long, J. L. Adams, G. Chan, K. S. Vaidya, T. A. Francis, K. Kikly, J. D. Winkler, C. M. Sung, C. Debouck, S. Richardson, M. A. Levy, W. E. DeWolf, P. M. Keller, T. Tomaszek, M. S. Head, M. D. Ryan, R. C. Haltiwanger, P. H. Liang, C. A. Janson, P. J. McDevitt, K. Johanson, N. O. Concha, W. Chan, S. S. Abdel-Meguid, A. M. Badger, M. W. Lark, D. P. Nadeau, L. J. Suva, M. Gowen and M. E. Nuttall, *J. Biol. Chem*., 2000, **275**, 16007.
400. K. Sol Church, J. Frenck and R. W. Mason, *Biochem. Biophys. Res. Commun*., 2000, **267**, 791.
401. D. S. Yamashita and R. A. Dodds, *Curr. Pharm. Design*, 2000, **6**, 1.
402. A. Nakajima, K. Kataoka, Y. Takata and N. H. Huh, *Biochem. J*., 2000, **349**, 689.
403. J. R. Somoza, H. U. Zhan, K. K. Bowman, L. Yu, K. D. Mortara, J. T. Palmer, J. M.

Clark and M. E. McGrath, *Biochemistry*, 2000, **39**, 12543.
404. G. Guncar, I. Klemencic, B. Turk, V. Turk, A. Karaoglanovic-Carmona, L. Juliano and D. Turk, *Struct. Folding and Design*, 2000, **8**, 305.
405. J. X. Song, P. Xu, H. Xiang Z. D. Su, A.C. Storer and F. Ni, *FEBS Lett*., 2000, **475**, 157.
406. T. Cierpicki, J. Bania and J. Otlewski, *Prot. Sci*., 2000, **9**, 976.
407. A. Yamamoto, K. Tomoo, H. Miyagawa, Y. Takaoka, S. Sumiya, K. Kitamura and T. Ishida, *Chem. Pharm. Bull*., 2000, **48**, 480.
408. Y. L. Guo, U. Kurz, J. E. Schultz, C. C. Lim, B. Wiederanders and K. Schilling, *FEBS Lett*., 2000, **469**, 203.
409. J. Guay, J. P. Falgueyret, A. Ducret, M. D. Percival and J. A. Mancini, *Eur. J. Biochem*., 2000, **267**, 6311.
410. C. J. Billington, P. Mason, M. C. Magny and J. S. Mort, *Biochem. Biophys. Res. Commun*., 2000, **276**, 924.
411. M. Horn, M. Pavlik, L. Doleckova, M. Baudys and M. Mares, *Eur. J. Biochem*., 2000, **267**, 3330.
412. A. Brinker, E. Weber, D. Stoll, J. Voigt, A. Muller, N. Sewald, G. Jung, K. H. Wiesmuller and P. Bohley, *Eur. J. Biochem*., 2000, **267**, 5085.
413. Y. Nagao, S. Sano, K. Morimoto, H. Kakegawa, T. Taketani, M. Shiro and N. Katunuma, *Tetrahedron Lett*., 2000, **41**, 2419.
414. H. S. Lee and K. J. Lee, *Peptides* 2000, **21**, 807.
415. N. Schaschke, I. Assfalg-Machleidt, T. Lassleben, C. P. Sommerhoff, L. Moroder and W. Machleidt, *FEBS Lett*., 2000, **482**, 91.
416. B. Walker, J. F. Lynas, M. A. Meighan and D. Bromme, *Biochem. Biophys. Res. Commun*., 2000, **275**, 401.
417. J. F. Lynas, S. J. Hawthorne and B. Walker, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1771.
418. N. Katunuma, A. Matsui, T. Inubushi, E. Murata, H. Kakegawa, Y. Ohba, D. Turk, V. Turk, Y. Tada and T. Asao, *Biochem. Biophys. Res. Commun*. 2000, **267**, 850.
419. X. N. Bi, T. S. Haque, J. Zhou, A. G. Skillman, B. Lin, C. E. Lee, I. D. Kuntz, J. A. Ellman and G. Lynch, *J. Neurochem*., 2000, **74**, 1469.
420. R. W. Marquis, *Annu. Rep. Med. Chem*., 2000, **35**, 309.
421. R. E. Dolle, *J. Combin. Chem*., 2000, **2**, 383.
422. M. A. Murcko, P. R. Caron and P. S. Charifson, *Annu. Rep. Med. Chem*., 1999, **34**, 297.
423. G. Falardeau, L. Chan, T. Stefanac, S. May, H. L. Jin and J. F. Lavallee, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2769.
424. A. Martinez, C. Gil, M. I. Abasolo, A. Castro, A. M. Bruno, C. Perez, C. Prieto and J. Otero, *J. Med. Chem*., 2000, **43**, 3218.
425. A. Martinez, C. Gil, C. Perez, A. Castro, C. Prieto, J. Otero, G. Andrei, R. Snoeck, J. Balzarini and E. De Clercq, *J. Med. Chem*., 2000, **43**, 3267.
426. L. Chan, T. Stefanac, N. Turcotte, Z. H. Hu, Y. M. Chen, J. Bedard, S. May and H. L. Jin, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1477.
427. M. Xian, Q. M. Wang, X. C. Chen, K. Wang and P. G. Wang, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2097.
428. P. S. Dragovich, R. Zhou, S. E. Webber, T. J. Prins, A. K. Kwok, K. Okano, S. A. Fuhrman, L. S. Zalman, F. C. Maldonado, E. L. Brown, J. W. Meador, A. K. Patick, C. E. Ford, M. A. Brothers, S. I. Binford, D. A. Matthews, R. A. Ferre and S. T. Worland, *Bioorg. Med. Chem. Lett*., 2000, **10**, 45.
429. S. H. Reich, T. Johnson, M. B. Wallace, S. E. Kephart, S. A. Fuhrman, S. T.

Worland, D. A. Matthews, T. F. Hendickson, F. Chan, J. Meador, R. A. Ferre, E. L. Brown, D. M. DeLisle, A. K. Patick, S. L. Binford and C. E. Ford, *J. Med. Chem.*, 2000, **43**, 1670.
430. R. D. Hill and J. C. Vederas, *J. Org. Chem.*, 1999, **64**, 9538.
431. A. Garozzo, C. C. C. Cutri, A. Castro, G. Tempera, F. Guerrara, M. C. Sarva and E. Geremia, *Antiviral Res.*, 2000, **45**, 199.
432. L. S. Zalman, M. A. Brothers, P. S. Dragovich, R. Zhou, T. J. Prins, S. T. Worland and A. K. Patick, *Antimicrobiol. Agents Chemoth.*, 2000, **44**, 1236.
433. I. B. Bersuker, S. Bahceci and J. E. Boggs, *J. Chem. Info Comp. Sci.*, 2000, **40**, 1363.
434. S. Hillaert and W. Van den Bossche, *J. Chromatogr. A*, 2000, **895**, 33.
435. D. L. Steer, R. A. Lew, P. Perlmutter, A. I. Smith and M.-I. Aguilar, *J. Pept. Sci.*, 2000, **6**, 470.
436. M. Azizi, C. Massien, A. Michaud and P. Corvol, *Hypertension*, 2000, **35**, 1226.
437. J. A. Robl, L. M. Simpkins and M. M. Asaad, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 257.
438. T. Quaschning, R. Corti, F. T. Ruschitzka, C. Noll and T. F. Luscher, *Deutsch. Med. Wochenschrift*, 2000, **125**, 499.
439. B. M. Loffler, *J. Cardiovas. Pharmacol.*, 2000, **35**, S79.
440. D. C. Fahnoe, G. D. Johnson, S. B. Herman and K. Ahn, *Arch. Biochem. Biophys.*, 2000, **373**, 385.
441. M. Letourneau, P. Roby, F. Tremblay, J. Carette and A. Fournier, *J. Cardiovas. Pharmacol.*, 2000, **36**, S28.
442. C. D. Wu, *Expert Opin. Therapeutic Patents*, 2000, **10**, 1653.
443. C. A. Fink, M. Moskal, F. Firooznia, D. Hoyer, D. Symonsbergen, D. C. Wei, Y. Qiao, P. Savage, M. E. Beil, A. J. Trapani and A. Y. Jeng, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2037.
444. S. De Lombaert, L. Blanchard, L. B. Stamford, J. Tan, E. M. Wallace, Y. Satoh, J. Fitt, D. Hoyer, D. Simonsbergen, J. Moliterni, N. Marcopoulos, P. Savage, M. Chou, A. J. Trapani and A. Y. Jeng, *J. Med. Chem.*, 2000, **43**, 488.
445. A. Y. Jeng, S. De Lombaert, M. E. Beil, C. W. Bruseo, P. Savage, M. Chou and A. J. Trapani, *J. Cardiovasc. Pharmacol.*, 2000, **36**, S36; A. J. Trapani, M. E. Beil, C. W. Bruseo, S. Lombaert and A. Y. Jeng, *ibid.*, 2000, **36**, S40.
446. A. J. Trapani, S. De Lombaert, M. E. Bell, C. W. Bruseo, P. Savage, M. Chou and A. Y. Jeng, *Life Sci.*, 2000, **67**, 1025.
447. K. Umekawa, H. Hasegawa, Y. Tsutumi, K. Sato, Y. Matsumura and N. Ohashi, *Jap. J. Pharmacol.*, 2000, **84**, 7.
448. B. C. Yoo, K. Aoki, Y. Xiang, L. R. Campbell, R. J. Hull, B. Xoconostle-Cazares, J. Monzer, J. Y. Lee, D. E. Ullmann and W. J. Lucas, *J. Biol. Chem.*, 2000, **275**, 35122.
449. H. S. Lee, W. Jin, S. G. Kang, Y. S. Hwang, Y. H. Kho and K. J. Lee, *J. Microb. Biotech.*, 2000, **10**, 81.
450. P. A. Wright, A. A. Rostom, C. V. Robinson and C. J. Schofield, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1219.
451. I. Nakanishi, T. Kinoshita, A. Sato and T. Tada, *Biopolymers*, 2000, **53**, 434.
452. I. Nakanishi, A. Fujikawa, K. Imai and A. Sato, *J. Pept. Res.*, 2000, **55**, 120.
453. H. H. Wasserman, J. H. Chen and M. D. Xia, *Helv. Chim. Acta*, 2000, **83**, 2607.
454. J. D. McBride, H. N. M. Freeman and R. J. Leatherbarrow, *Eur. J. Biochem.*, 1999, **266**, 403.
455. P. G. Baraldi, B. Cacciari, R. Romagnoli and G. Spatullo, *Arzneimittel-Forsch.-Drug.Res.* 1999, **49**, 997.
456. R. C. Wilmouth, Y. H. Li, P. A. Wright, T. D. W. Claridge, R. T. Aplin and C. J.

Schofield, *Tetrahedron*, 2000, **56**, 5729.
457. K. Achilles, M. Schneider, T. Schirmeister and H. H. Otto, *Pharmazie*, 2000, **55**, 798; K. Achilles, T. Schirmeister and H. H. Otto, *Arch. Pharm.* (*Weinheim*, *Ger.*), 2000, **333**, 243.
458. B. Macchia, D. Gentili, M. Macchia, F. Mamone, A. Martinelli, E. Orlandini, A. Rossello, G. Cercignani, R. Pierotti, M. Allegretti, C. Asti and G. Caselli, *Eur. J. Med. Chem.*, 2000, **35**, 53.
459. M. A. Laborde and O. A. Masceratti, *Molecules*, 2000, **5**, 655.
460. M. Gutschow, L. Kuerschner, U. Neumann, M. Pietsch, R. Loser, N. Koglin and K. Eger, *J. Med. Chem.*, 1999, **42**, 5437.
461. J. E. Kerrigan, M. C. Walters, K. J. Forrester, J. B. Crowder and L. J. Christopher, *Bioorg. Med. Chem.*, 2000, **10**, 27.
462. N. Terrett, *Drug Disc. Today*, 2000, **5**, 124.
463. E. Perola, K. Xu, T. M. Kollmeyer, S. H. Kaufmann, F. G. Prendergast and Y. P. Pang, *J. Med. Chem.*, 2000, **43**, 401.
464. E. Giraud, C. Luttmann, F. Lavelle, J. F. Riou, P. Mailliet and A. Laoui, *J. Med. Chem.*, 2000, **43**, 1807.
465. L. Carlacci, *J. Comp.-Aided Mol. Design*, 2000, **14**, 369.
466. T. J. Zahn, M. Eilers, Z. M. Guo, M. B. Ksebati, M. Simon, J. D. Scholten, S. O. Smith, *J. Am. Chem. Soc.*, 2000, **122**, 7153.
467. K. A. H. Chehade, D. A. Andres, H. Morimoto and H. P. Spielmann, *J. Org. Chem.*, 2000, **65**, 3027.
468. S. K. Tahir, W. Z. Gu, H. C. Zhang, J. Leal, J. Y. Lee, P. Kovar, B, Saeed, S. P. Cherian, E. Devine, J. Cohen, R. Warner, Y. C. Wang, D. Stout, D. L. Arendsen, S. Rosenberg and S. C. Ng, *Eur. J. Cancer*, 2000, **36**, 1161.
469. L. H. Cohen, E. Pieterman, R. E. W. vanLeeuwen, M. Overhand, B. E. A. Burm, G. A. van der Marel and J. H. van Boom, *Biochem. Pharmacol.*, 2000, **60**, 1061.
470. J. T. Hunt, C. Z. Ding, R. Batorsky, M. Bednarz, R. Bhide, Y. Cho, S. Chong, S. Chao, J. Gullo-Brown, P. Guo, S. H. Kim, F. Y. F. Lee, K. Leftheris, A. Miller, T. Mitt, M. Patel, B. A. Penhallow, C. Ricca, W. C. Rose, R. Schmidt, W. A. Slusarchyk, G.Vite and V. Manne, *J. Med. Chem.*, 2000, **43**, 3587.
471. M. A. Kothare, J. Ohkanda, J. W. Lockman, Y. Qian, M. A. Blaskovich, S. M. Sebti and A. D. Hamilton, *Tetrahedron*, 2000, **56**, 9833.
472. C. Z. Ding, J. T. Hunt, C. Ricca and V. Manne, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 273.
473. C. J. Dinsmore, C. B. Zartman, W. F. Baginsky, T. J. O'Neill, K. S. Koblan, I. W. Chen, D. A. McLoughlin, T. V. Olah and J. R. Huff, *Org. Lett.*, 2000, **2**, 3473.
474. M. Schlitzer, M. Bohm, I. Sattler and H. M. Dahse, *Bioorg. Med. Chem.*, 2000, **8**, 1991.
475. M. Schlitzer, M. Bohm and I. Sattler, *Bioorg. Med. Chem.*, 2000, **8**, 2399.
476. M. Schlitzer and I. Sattler, *Eur. J. Med. Chem.*, 2000, **35**, 721.
477. C. E. O'Connell, C. A. Rowell, K. Ackermann, A. M. Garcia, M. D. Lewis and J. J. Kowalczyk, *Chem. Pharm. Bull.*, 2000, **48**, 740.
478. T. Hirose, T. Sunazaka, Z. M. Tian, M. Handa, R. Uchida, K. Shiomi, Y. Harigaya and S. Omura, *Heterocycles*, 2000, **53**, 777.
479. F. Y. F. Lee, M. Arico, C. Burke, J. Clark, K. Fager, S. Rex, C. Ricca, R. Smykla, C. VanDeren, M. Wen, W. Rose and V. Manne, *Clin. Cancer. Res.*, 2000, **6**, 401.
480. B. Silbermann, C. Yousry, D. Salmon-Ceron, *Ann. Med. Interne*, 2000, **151**, 268.
481. D. P. Fairlea, J. D. A. Tyndall, R. C. A. Reid, A. K. Wong, G. Abbenante, M. J. Scanlon, D. R. March, D. A. Bergmann, C. L. L. Chai and B. A. Burkett, *J. Med.*

Chem., 2000, **43**, 1271.
482. E. Alonso, E. A. Alonso, A. Solis and C. Del Pozo, *Synlett*, 2000, 698.
483. E. J. Stoner, A. J. Cooper, D. A. Dickman, L. Kolackowski, J. E. Lallaman, J.-H. Liu, P. A. Oliver-Shaffer, K. M. Patel, J. P. Paterson Jr., D. J. Plata, D. A. Riley, L. H. Sham, P. J. Stengel and J.-H. J. Tien, *Org. Process Res. Dev*., 2000, **4**, 264.
484. J. L. Kraus, M. Bouygues, J. Courcambeck and J. C. Chermann, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2023.
485. J. Wachtmeister, A. Muhlman, B. Classon, I. Kvarnstrom, A. Hallberg and B. Samuelsson, *Tetrahedron*, 2000, **56**, 3219.
486. A. Spaltenstein, M. R. Almond, W. J. Bock and D. G. Cleary, E. S. Furfine, R. J. Hazen, W. M. Kazmierski, F. G. Salituro, R. D. Tung and L.L. Wright, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1159.
487. T. Mimoto, N. Hattori, H. Takaku, S. Kisanuki, T. Fukazawa, K. Terashima, R. Kato, S. Nojima, S. Misawa, T. Ueno, J. Imai, H. Enomoto, S. Tanaka, H. Sakikawa, M. Shintani, H. Hayashi and Y. Kiso, *Chem. Pharm. Bull*., 2000, **48**, 1310.
488. M. D. Shultz, M. J. Bowman, Y.-W. Ham, X. Zhao, G. Tora and J. Chmielewski, *Angew. Chem. Int. Ed*., 2000, **39**, 2710.
489. H. Matsumoto, T. Hamakawi, H. Ota, T. Kimura, T. Goto, K. Sano, Y. Hayashi and Y. Kiso, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1227.
490. G. Priem, L. Rocheblave, C. De Michelis, J. Courcambeck and J. L. Kraus, *J. Chem. Soc. Perkin Trans. 1*, 2000, 819.
491. Y. S. Lee, Y. S. Lee, J. Y. Lee, S. N. Kim, C. K. Lee and H. Park, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2625.
492. P. L. Beaulieu, P. C. Anderson, D. R. Cameron, G. Croteau, V. Gorys, C. Grand-Maitre, D. Lamarre, F. Laird, W. Paris, L. Plamondon, F. Soucy, D. Thilbeault, D. Wernic, C. Yoakim, S. Pav and L. Tong, *J. Med. Chem*., 2000, **43**, 1094.
493. B. D. Dorsey, C. McDonough, S. L. McDaniel, R. B. Levin, C. L. Newton, J. M. Hoffman, P. L. Darke, J. A. Zugay-Murphy, E. A. Emini, W. A. Schleif, D. B. Olsen, M. W. Stahlhut, C. A. Rutkowski, L. C. Kuo, J. H. Lin, I. W. Chen, S. R. Michelson, M. K. Holloway, J. R. Huff and J. P. Vacca, *J. Med. Chem*., 2000, **43**, 3386.
494. A. Datta and G. Veeresa, *J. Org. Chem*., 2000, **65**, 7609.
495. J. M. Dougherty, D. A. Probst, R. E. Robinson, J. D. Moore, T. A. Klein, K. A. Snelgrove and P. R. Hanson, *Tetrahedron*, 2000, **56**, 9781.
496. K. T. Sprott and P. R. Hanson, *J. Org. Chem*., 2000, **65**, 7913.
497. J. W. Skiles, L. G. Monovich and A. Y. Jeng, *Annu. Rep. Med. Chem*., 2000, **35**, 167.
498. S. Hanessian, N. Moitessier and S. Wilmouth, *Tetrahedron*, 2000, **56**, 7643.
499. J. Buchardt, C. B. Schiodt, C. Krog-Jensen, J.-M. Delaisse, N. T. Foged and M. Meldal, *J. Comb. Chem*., 2000, **2**, 624.
500. M. Y. Cheng, B. De, S. Pikul, N. G. Almstead, M. G. Natchus, M. V. Anastasio, S. J. McPhail, C. E. Snider, Y. O. Taiwo, L. Y. Chen, C. M. Dunaway, F. Gu, M. E. Dowty, G. E. Mieling, M. J. Janusz and S. Wang-Weigand, *J. Med. Chem*., 2000, **43**, 369.
501. R. P. Robinson, E. R. Laird, J. F. Blake, J. Bordner, K. M. Donahue, L. L. Lopresti-Morrow, P. G. Mitchell, M. R. Reese, L. M. Reeves, E. J. Stam and S. A. Yocum, *J. Med. Chem*., 2000, **43**, 2293.
502. A. Scozzafava and C. T. Supuran, *J. Med. Chem*., 2000, **43**, 1858.
503. A. Scozzafava and C. T. Supuran, *J. Med. Chem*., 2000, **43**, 3677.
504. A. Scozzafava, M. A. Ilies, G. Manole and C. T. Supuran, *Eur. J. Pharm. Sci*., 2000, **11**, 69.
505. A. Simeon, H. Emonard, W. Hornebeck and F. X. Maquart, *Life Sci*., 2000, **67**,

2257.
506. T. L. McCready, B. F. Islam, F. J. Scmitz, H. A. Luu, J. F. Dawson and C. F. B. Holmes, *J. Biol. Chem.*, 2000, **275**, 4192.
507. Y. Katayose, M. Li, S. W. K. Al-Murrani, S. Shenolikar and Z. Damuni, *J. Biol. Chem.*, 2000, **275**, 9209.
508. K. X. Huang and H. K. Paudel, *Proc. Nat. Acad. Sci. USA*, 2000, **97**, 5824.
509. J. Yang, T. D. Hurley, A. A. DePaoli-Roach, *J. Biol. Chem.*, 2000, **275**, 22635.
510. H. Huang, Y. Chen, L. Tsai, H. Wang, F. M. Lin, A. Horiuchi, P. Greengard, A. C. Nairn, M. S. Shiao and T. H. Lin, *J. Biomol. NMR*, 2000, **17**, 359.
511. I. C. Gonzalez and C. J. Forsyth, *J. Am. Chem. Soc.*, 2000, **122**, 9099.
512. P. Wipf, D. C. Aslan, D. K. Luci, E. C. Southwick and J. S. Lzo, *Biotech. BioEng.*, 2000, **71**, 58.
513. P. S. Savle, T. E. Shelton, C. A. Meadows, M. Potts, R. D. Gandour and P. J. Kennelly, *Arch. Biochem. Biophys.*, 2000, **376**, 439.
514. C. W. Laidley, W. G. Dauben, Z. R. Guo, J. Y. L. Lam and J. E. Casida, *Bioorg. Med. Chem.*, 1999, **7**, 2937.
515. A. Garcia, S. Cereghini and E. Sontag, *J. Biol. Chem.*, 2000, **275**, 9385.
516. M. Ianosi-Irimie and P. Deutsch, *Pharmazie*, 2000, **55**, 629.
517. D. A. Sandham, R. J. Taylor, J. S. Carey and A. Fässler, *Tetrahedron Lett.*, 2000, **41**, 10091.
518. B. Simoneau, P. Lavallee, M. Bailey, J. S. Duceppe, C. Grand-Maetre, L. Grenier, W. W. Ogilvie, M. A. Poupart and B. Thavonekham, *Can. J. Chem.*, 2000, **78**, 739.
519. J. Rahuel, V. Rasetti, J. Maibaum, H. Rueger, R. Goschke, N. C. Cohen, S. Stutz, F. Cumin, W. Fuhrer, J. M. Wood and M. G. Grutter, *Chem. Biol.*, 2000, **7**, 493.
520. G. Lefevre and S. Gauron, *J. Chromatogr. B.*, 2000, **738**, 129,
521. N. B. Cronin, M. O. Badasso, I. J. Tickle, T. Dreyer, D. J. Hoover, R. L. Rosati, C. C. Humblet, E. A. Lunney and J. B. Cooper, *J. Mol. Biol.*, 2000, **303**, 745.
522. C. L. Moore, D. D. Leatherwood, T. S. Diehl, D. J. Selkoe and M. S. Wolfe, *J. Med. Chem.*, 2000, **43**, 3434.
523. C. Pesenti, A. Arnone, A. M. Aubertin, P. Bravo, M. Frigerio, W. Panzeri, S. Schmidt, F. Viani and M. Zanda, *Tetrahedron Lett.*, 2000, **41**, 7239.
524. M. A. Kastenholz, M. Pastor, G. Cruciani, E. E. J. Haaksma and T. Fox, *J. Med. Chem.*, 2000, **43**, 3033.
525. M. Salzet, V. Chopin, J. L. Baert, I. Matias and J. Malecha, *J. Biol. Chem.*, 2000, **275**, 30774.
526. J. J. Sion-Usakiewicz, J. Sivaraman, Y. G. Li, M. Cygler, Y. Konishi, *Biochemistry*, 2000, **39**, 2384.
527. P. Betschmann, C. Lerner, S. Sahli, U. Obst and F. Diederich, *Chimia*, 2000, **54**, 633.
528. U. Obst, P. Betschmann, C. Lerner, P. Seiler, F. Diedrich, V. Gramlich, L. Weber, D. W. Banner and P. Schonholzer, *Helv. Chim. Acta*, 2000, **83**, 855.
529. D. Noteberg, J. Branalt, I. Kvarnstrom, M. Linschoten, D. Musil, J. E. Nystrom, G. Zuccarello and B. Samuelsson, *J. Med. Chem.*, 2000, **43**, 1705.
530. K. Lee, W. H. Jung, M. Kang and S. H. Lee, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2775.
531. C. A. Coburn, D. M. Rush, P. D. Williams, C. Homnick, E. A. Lyle, S. D. Lewis, B. J. Lucas, J. M. DiMuzio-Mower, M. Juliano, J. A. Krueger, K. Vastag, I. W. Chen and J. P. Vacca, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1069.
532. A. von Matt, C. Ehrhardt, P. Burkhard, R. Metternich, M. Walkinshaw and C. Tapparelli, *Biorg. Med. Chem.*, 2000, **8**, 2291.
533. R. M. Soll, T. Lu, B. Tomczuk, C. R. Illig, C. Fedde, S. Eisennagel, R. Bone, L.

Murphy, J. Spurlino and F. R. Salemme, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1.
534. T. B. Lu, R. M. Soll, C. R. Illig, R. Bone, L. Murphy, J. Spurlino, F. R. Salemme and B. E. Tomczuk, *Bioorg. Med. Chem. Lett*., 2000, **10**, 79.
535. T. B. Lu, B. Tomczuk, R. Bone, L. Murphy, F. R. Salemme and R. M. Soll, *Bioorg. Med. Chem. Lett*., 2000, **10**, 83.
536. U. Baettig, L. Brown, D. Brundish, C. Dell, A. Furzer, S. Garman, D. Janus, P. D. Kane, G. Smith, C. V. Walker, X. L. Cockcroft, J. Ambler, A. Mitchelson, M. D. Talbot, M. Tweed and N. Wills, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1563.
537. J. Hayler, P. D. Kane, D. LeGrand, F. Lugrin, K. Menear, R. Price, M. Allen, X. L. Cockcroft, J. Ambler, K. Butler, K. Dunnet, A. Mitchelson, M. Talbot, M. Tweed and N. Wills, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1567.
538. C. T. Supuran, A. Scozzafava, F. Briganti and B. W. Clare, *J. Med. Chem*., 2000, **43**, 1793; B. W. Clare, A. Sczzafava, F. Briganti, B. Iorga and C. T. Supuran, *J. Enz. Inhib*., 2000, **15**, 235.
539. L. S. Narasimhan, J. R. Rubin, D. R. Holland, J. S. Plummer, S. T. Rapundalo, J. E. Edmunds, Y. St-Denis, M. A. Siddiqui and C. Humblet, *J. Med. Chem*., 2000, **43**, 361.
540. N. Y. Chirgadze, D. J. Sall, S. L. Briggs, D. K. Clawson, M. Zhang, G. F. Smith and R. W. Schevitz, *Protein Sci*., 2000, **9**, 29.
541. D. J. Sall, D. L. Bailey, J. A. Bastian, J. A. Buben, N. Y. Chirgadze, A. C. Clemens-Smith, M. L. Denney, M. J. Fisher, D. D. Giera, D. S. Gifford-Moore, R. W. Harper, L. M. Johnson, V. J. Klimowski, T. J. Kohn, H. S. Lin, J. R. McCowan, A. D. Palkowitz, M. E. Richett, G. F. Smith, D. W. Snyder, K. Takeuchi, J. E. Toth and M. S. Zhang, *J. Med. Chem*., 2000, **43**, 649.
542. K. Takeuchi, T. J. Kohn, R. W. Harper, H. S. Lin, D. S. Gifford-Moore, M. E. Richett, D. J. Sall, G. F. Smith and M. S. Zhang, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1199.
543. K. Takeuchi, J. A. Bastian, D. S. Gifford-Moore, R. W. Harper, S. C. Miller, T. J. Mullaney, D. J. Sall, G. F. Smith, M. S. Zhang and M. J. Fischer, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2347.
544. T. Steinmetzer, M. Batdordshjin, F. Pineda, L. Seyfarth, A. Vogel, S. Reissmann, J. Hauptmann and J. Sturzebecher, *Biol. Chem*. 2000, **381**, 603.
545. D. Green, S. Elgendy, G. Patel, E. Skordalakes, C. A. Goodwin, M. F. Scully, V. V. Kakkar and J. J. Deadman, *Phosph. Sulf. Silicon Relat. Elem*., 2000, **156**, 151.
546. L. Leblond, B. Grouix, C. Boudreau, Q. Yang, M. A. Siddiqui and P. D. Winocour, *Thromb. Res*., 2000, **100**, 195.
547. J. E. Semple, T. D. Owens, K. Nguyen and O. E. Levy, *Org. Lett*., 2000, **2**, 2769.
548. Q. Han, C. Dominguez, P. F. W. Stouten, J. M. Park, D. E. Duffy, R. A. Galemmo, K. A. Rossi, R. S. Alexander, A. M. Smallwood, P. C. Wong, M. M. Wright, J. M. Luettgen, R. M. Knabb and R. R. Wexler, *J. Med. Chem*., 2000, **43**, 4398.
549. D. K. Heron, T. Goodson, M. R. Wiley, L. C. Weir, J. A Kyle, Y. K. Yee, A. L. Tebbe, J. M. Tinsley, D. Mendel, J. J. Masters, B. Franciskovich, J. S. Sawyer, D. W. Beight, A. M. Ratz, G. Milot, S. E. Hall, V. J. Klimoswski, J. H. Wikel, B. J. Eastwood, R. D. Towner, D. S. Gifford-Moore, T. J. Craft and G. F. Smith, *J. Med. Chem*., 2000, **43**, 859.
550. Y. K. Yee, A. L. Tebbe, J. H. Linebarger, D. W. Beight, T. J. Craft, D. Gifford-Moore, T. Goodson, D. K. Heron, V. J. Klimoswski, J. A. Kyle, J. S. Sawyer, G. F. Smith, J. M. Tinsley, R. D. Towner, L. Weir and M. R. Wiley, *J. Med. Chem*., 2000, **43**, 873.
551. C. K. Marlowe, U. Sinha, A. C. Gunn and R. M. Scarborough, *Bioorg. Med. Chem.*

Lett., 2000, **10**, 13.
552. J. E. Semple, O. E. Levy, N. K. Minami, T. D. Owens and D. V. Siev, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2305.
553. J. Cacciola, J. M. Fevig, P. F. W. Stouten, R. S. Alexander, R. M. Knabb and R. R. Wexler, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1253.
554. S. Maignan, J. P. Guilloteau, S. Pouzieux, Y. M. Choi-Sledeski, M. R. Becker, S. I. Klein, W. R. Ewing, H. W. Pauls, A. P. Spada and V. Mikol, *J. Med. Chem*., 2000, **43**, 3226.
555. R. Smith, A. Jenkins, A. Lourbakos, P. Thomson, V. Ramakrishnan, J. Tomlinson, U. Deshpande, D. A. Johnson, R. Jones, E. J. Mackie and R. N. Pike, *FEBS Letters*, 2000, **484**, 285.
556. M. Steinhoff, N. Vergnolle, S. H. Young, M. Tognetto, S. Amadesi, H. S. Ennes, M. D. Hollenberg, J. L. Wallace, G. H. Caughey, S. E. Mitchell, L. M. Williams, P. Gepetti, E. A. Mayer and N. W. Bunnett, *Nature Medicine*, 2000, **6**, 151.
557. A. K. Alm, R. Gagnemo-Persson, T. Sorsa and J. Sundelin, *Biochem. Biophys. Res. Commun*., 2000, **275**, 77.
558. A. Kawabata, N. Morimoto, H. Nishikawa, R. Kuroda, Y. Oda and K. Kakehi, *Biochem. Biophys. Res. Commun*., 2000, **270**, 298.
559. S. Miyata, N. Koshikawa, H. Yasimitsu and K. Miyazaki, *J. Biol. Chem*., 2000, **275**, 4592.
560. A. Hijikata-Okunomiya, Y. Tamao, R. Kikumoto and S. Okamoto, *J. Biol. Chem*., 2000, **275**, 18995.
561. C. Tirauppathi, W. H. Yan, R. Sandoval, T. Naqvi, A. N. Pronin, J. L. Benovic and A. B. Malik, *Proc. Natl. Acad. Sci., USA*, 2000, **97**, 7440.
562. S. J. Chung and D. H. Kim, *Bioorg. Med. Chem*., 2000, **9**, 185.
563. Abbott, D. M. T. Yu, E. Woolatt, G. R. Sutherland, G. W. McGaughan and M. D. Gorrell, *Eur. J. Biochem*., 2000, **267**, 6140.
564. Y. H. Li, T. MaedaT. Yamane and I. Ohkubo, *Biochem. Biophys. Res. Commun*., 2000, **276**, 553.
565. I. De Meester, C. Durinx, G. Bal, P. Proost, S. Struyf, F. Goossens, K. Augustyns and S. Scharpe, *Cell. Peptidases in Immun. Funct. and Diseases*, 2000, **477**, 67.
566. A. Stockel-Maschek, B. Stiebitz, I. Born, J. Faust, W. Mogelin and K. Neubert, *Cell. Peptidases in Immun. Funct. and Diseases*, 2000, **477**, 117.
567. C. Mrestani-Klaus, A. Fengler, J. Faust, W. Brandt, S. Wrenger, D. Reinhold, S. Ansorge and K. Neubert, *Cell. Peptidases Immun. Funct. Diseases*, 2000, **477**, 125.
568. M. Arndt, D. Reinhold, U. Lendeckel, A. Spiess, J. Faust, K. Neubert and S. Ansorge, *Cell. Peptidases Immun. Funct. Diseases*, 2000, **477**, 139.
569. I. Hermecz and K. Kanai, *Farmaco*, 2000, **55**, 188.
570. H. J. Lee, I. K. Rhee, K. B. Lee, I. D. Yoo and K. S. Song, *J. Antibiotics*, 2000, **53**, 714.
571. W. Fan, Y. Tezuka and S. Kadota, *Chem. Pharm. Bull*., 2000, **48**, 1055.
572. S. R. Hubbard and J. H. Till, *Ann. Rev. Biochem*., 2000, **69**, 373.
573. K. Kim, K. Parang, O. D. Lau and P. A. Cole, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1263.
574. M. Oblak, M. Randic and T. Solmajer, *J. Chem. Info. Comp. Sci*., 2000, **40**, 994.
575. B. S. Gaul, M. L. Harrison, R. L. Geahlen, R. K. Burton and C. B. Post, *J. Biol. Chem*., 2000, **275**, 16174.
576. G. Wells, A. Seaton and M. F. G. Stevens, *J. Med. Chem*., 2000, **43**, 1550.
577. K. H. Kim, K. Parang, O. D. Lau and P. A. Cole, *Biochemistry*, 2000, **39**, 78.
578. G. Radau, *Pharmazie*, 2000, **55**, 555.

579. A. C. Frobius, M. R. Kanost, P. Gotz and A. Vilcinskas, *Eur. J. Biochem.*, 2000, **267**, 2046.
580. T. Cierpicki, J. Bania and J. Otlewski, *Protein Sci.*, 2000, **9**, 976.
581. N. Valls, M. Lopez-Canet, M. V. Vallibera and J. Bonjoch, *J. Am. Chem. Soc.*, 2000, **122**, 11248.
582. N. J. Ede, S. N. Eagle, G. Wickham, A. M. Bray, B. Warne, K. Shoemaker and S. Rosenberg, *J. Pept. Sci.*, 2000, **6**, 11.
583. P. Imming, B. Klar and D. Dix, *J. Med. Chem.*, 2000, **43**, 4328.
584. J. D. McBride, H. N. Freeman and R. J. Leatherbarrow, *J. Pept. Sci.*, 2000, **6**, 446.
585. S. J. Shuttleworth, D. Nasturica, C. Gervais, M. A. Siddiqui, R. F. Rando and N. Lee, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2501.
586. P. Jakobsen, B. R. Pedersen and E. Persson, *Bioorg. Med. Chem.*, 2000, **8**, 2095.
587. P. Jakobsen, A. M. Horneman and E. Persson, *Bioorg. Med. Chem.*, 2000, **8**, 2803.
588. L. P. Masic and D. Kikelj, *Tetrahedron Lett.*, 2000, **41**, 5589.
589. P. D. Edwards, R. C. Mauger, K. Cottrell, F. X. Morris, K. K. Pine, M. A. Sylvester, C. W. Scott and S. T. Furlong, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2291.
590. Y. Aoyama, M. Uenaka, T. Konoike, Y. Iso, Y, Nishitani, A. Kanda, N. Naya and M. Nakajima, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2397.
591. Y. Aoyama, M. Uenaka, T. Konoike, Y. Iso, Y, Nishitani, A. Kanda, N. Naya and M. Nakajima, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2403.
592. Y. Hayashi, K. Iijima, J. Katada and Y. Kiso, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 199.
593. K. D. Rice, A. R. Gangloff, E. Y. L. Kuo, J. M. Dener, V. R. Wang, R. Lum, W. S. Newcomb, C. Havel, D. Putman, L. Cregar, M. Wong and R. L. Warne, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2357.
594. K. D. Rice, V. R. Wang, A. R. Gangloff, E. Y. L. Kuo, J. M. Dener, W. S. Newcomb, W. B. Young, D. Putman, L. Cregar, M. Wong and P. J. Simpson, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2361.
595. C. S. V. Houge-Frydrych, M. L. Gilpin, P. W. Skett and J. W. Tyler, *J. Antibiotics*, 2000, **53**, 364.
596. C. S. V. Houge-Frydrych, S. A. Readshaw and D. J. Bell, *J. Antibiotics*, 2000, **53**, 351.
597. M. G. Banwell, C. F. Crasto, C. J. Easton, A. K. Forrest, T. Karoli, D. R. March, L. Mensah, M. R. Nairn, P. J. O'Hanlon, M. D. Oldham and W. Yue, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2263.
598. K. Obi, J.-I. Uda, K. Iwase, O. Sugimoto, H. Ebisu and A. Matsuda, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1451.
599. C. T. Supuran and A. Scozzafava, *Eur. J. Pharm. Sci.*, 2000, **10**, 67.
600. A. Ovens, J. A. Joule and K. E. Kadler, *J. Pept. Sci.*, 2000, **6**, 489.
601. G. Loidl, H.-J. Musiol, M. Groll, R. Huber and L. Moroder, *J. Pept. Sci.*, 2000, **6**, 36.
602. H. Huang, P. Mortasek, L. J. Roman and R. B. Silverman, *J. Med. Chem.*, 2000, **43**, 2938.
603. D. Ma and Q. Wu, *Tetrahedron Lett.*, 2000, **41**, 9089.
604. Y. Okamoto, M. Ojika, S. Kato and Y. Sakagami, *Tetrahedron*, 2000, **56**, 5813.
605. D. Isshikawa and T. Taki, *Trends in Glycosci. Glycotech.*, 1999, **11**, 277.
606. F. Viti, M. A. Bumke, M. Silacci, L. Zardi and D. Neri, *Chimia*, 2000, **54**, 678.
607. V. V. Ivanenkov and A. G. Menon, *Biochem. Biophys. Res. Commun.*, 2000, **276**, 251.
608. K. W. Chan, P. L. Lim, F. C. H. Tam, E. T. S. Li and B. L. Lim, *J. Pept. Res.*, 2000, **55**, 318.
609. N. Venkatesh, S. H. Im, M. Balass, S. Fuchs and E. Katchalski-Katzir, *Proc. Nat.*

Acad. Sci. USA, 2000, **97**, 761.
610. J. Ay, T. Keitel, G. Kuttner, H. Wessner, C. Scholz, M. Hahn and W. Hohne, *J. Mol. Biol.*, 2000, **301**, 239.
611. M. Vidal, W. Q. Liu, B. P. Roques and C. Garbay, *Clin. Cancer Res.*, 2000, **6**, 145.
612. D. Kampa and J. Burnside, *Biochem. Biophys. Res. Commun.*, 2000, **278**, 175.
613. J. Y. Nam, H. K. Kim, J. Y. Kwon, M. Y. Han, K. H. Son, U. C. Lee, J. D. Choi and B. M. Kwon, *J. Nat. Prod.*, 2000, **63**, 1303.
614. H. K. Kim, J. Y. Nam, M. Y. Han, K. H. Son, J. D. Choi, B. M. Kwon, H. L. Takusagawa, Y. F. Huang and F. Takusagawa, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1455.
615. P. Furet, G. Caravatti, A. A. Denholm, A. Faessler, H. Fretz, C. Garcia-Echeverria, B. Gay, E. Irving, N. J. Press, J. Rahuel, J. Schoepfer and C. V. Walker, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2337.
616. C. V. Walker, G. Caravatti, A. A. Denholm, J. Egerton, A. Faessler, P. Furet, C. Garcia-Echeverria, B. Gay, E. Irving, K. Jones, A. Lambert, N. J. Press and J. Woods, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2343.
617. W. C. Shakespeare, R. S. Bohacek, M. D. Azimioara, K. J. Macek, G. P. Luke, D. C. Dalgarno, M. H. Hatada, X. D. Lu, S. M. Violette, C. Bartlett and T. K. Sawyer, *J. Med. Chem.*, 2000, **43**, 3815.
618. Y. Gao, L. Wu, J. H. Luo, R. Guo, D. Yang, Z.-Y. Zhang and T. R. Burke Jr., *Bioorg. Med. Chem. Lett.*, 2000, **10**, 923.
619. Y. Gao, J. Luo, Z. J. Yao, R. B. Guo, H. Zou, J. Kelley, J. H. Voigt, D. J. Yang and T. R. Burke Jr., *J. Med. Chem.*, 2000, **43**, 911.
620. C. A. Metcalf, C. J. Eyermann, R. S. Bohacek, C. A. Haraldson, V. M. Varkhedkar, B. A. Lynch, C. Bartlett, S. M. Violette and T. K. Sawyer, *J. Comb. Chem.*, 2000, **2**, 305.
621. Y. C. Lou, F. D. T. Lung, M. T. Pai, S. R. Tzeng, S. Y. Wei, P. P. Roller and J. W. Cheng, *Arch. Biochem. Biophys.*, 1999, **372**, 309.
622. R. Phiosof-Oppenheimer, C. S. Hampe, K. Slessinger, M. Fridkin and I. Pecht, *Eur. J. Biochem.*, 2000, **267**, 703.
623. D. G. Liu, Z. J. Yao, Y. Gao and T. R. Burke Jr., *Org. Prep. Proc. Int.*, 2000, **32**, 197.
624. J. T. Nguyen, M. Porte, M. Amoui, W. T. Miller, R. N. Zuckermann and W. A. Lim, *Chemistry and Biology*, 2000, **7**, 463.
625. F. Cordier, C. Y. Wang, S. Grzesiek and L. K. Nicholson, *J. Mol. Biol.*, 2000, **304**, 497.
626. S. Macholl, I. Sack, H. H. Limbach, J. Pauli, M. Kelly and C. Buntkowsky, *Mag. Res in Chem.*, 2000, **38**, 596.

4
Cyclic, Modified and Conjugated Peptides

BY JOHN S. DAVIES

1 Introduction

To maintain continuity within volumes of these reports, the sub-divisions of this chapter have once again been kept very similar to previous volumes. Hopefully this will aid researchers reviewing specific sections on an annual basis. The harvesting of papers for this chapter has once again been greatly aided by two abstracting services, CA Selects[1] on Amino Acids, Peptides and Proteins (up to Issue 12, June 2001), and the index pages of Journals *via* the Web of Science databases[2] on the Internet. A compilation of papers presented at the 16th American Peptide Symposium at Minneapolis has been published,[3] but as in the past the contents of conference reports are not reviewed here until they appear as refereed papers.

The number of papers compiled in this chapter falls into recent averages, with glycopeptides and cyclodepsipeptides again vieying for being the most popular sub-sections. Marine natural products have become an expanding source of cyclic peptides and cyclodepsipeptides, as reflected in a review[4] of the literature up to 1999. Some of the chapters in a useful practical book[5] represent areas of direct interest to this chapter.

2 Cyclic Peptides

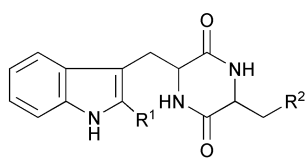
2.1 General Considerations. – Methods of synthesis and the making of libraries of head-to-tail cyclic peptides and cyclic pseudopeptides have been reviewed.[6] The application of the orthogonal ligation strategy to cyclic peptides, utilising unprotected peptides from biosynthetic sources, has also been reviewed.[7] A specialist review written by the main exponent of the use of 1,3-oxazol-5(4*H*)-ones, in the synthesis of macrolides, cyclodepsipeptides and cyclopeptides, has also appeared.[8]

Two side reactions[9] seem to accompany the use of the Dmab (4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl ester) as a protecting group for glutamyl in the synthesis of side chain to side chain cyclised peptides. Pyroglutamyl formation and the formation of a glutamyl 4-aminobenzyl ester account for the problems. The use of allyl esters provided a successful

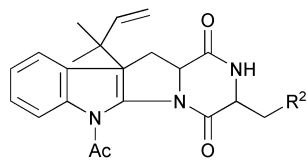
Amino Acids, Peptides and Proteins, Volume 33

answer. Model cyclic disulfide-containing prolyl peptides show an enhancement[10] in the *cis–trans* isomerisation at the acyl-Pro bonds. It is proposed that this enhancement provides experimental evidence for the intramolecular H-bonding mechanism for FK-506-binding protein-catalysed *cis–trans* isomerisation of the Xaa-Pro bond in proteins. Computational studies[11] on cyclo [(Phe-D-Ala)$_n$] (**1**) and cyclo [(Phe-D-MeAla)$_n$] (**2**) have shown that with $n = 3$, 4, 5 or 6, compounds (**1**) form nanotubes by self-assembly, but in (**2**) with $n = 3$, 5 or 6, no disk-like conformations were formed.

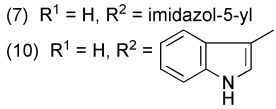
2.2 Cyclic Dipeptides (Dioxopiperazines). – Fungi of the species *Penicillium piscarium* produce[12] a series of dioxopiperazine alkaloids having the formulae (**3–9**), while *P. fellutanum* produces[13] the dioxopiperazines (**10–13**). The trichlorinated dysamide U (**14**) can now be added to the other seven known dioxopiperazines isolated and identified[14] from the sponge *Dysidea* sp., while the root of *Psammosilene tunicoides* has yielded[15] four new natural products, cyclo(Ala-Ala), cyclo(Ala-Val), cyclo(Ala-Leu) and cyclo(Ala-Ile).



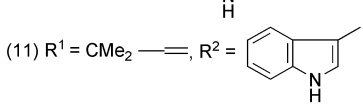
(3) R^1 = H, R^2 = Ph
(6) R^1 = H, R^2 = indol-3-yl
(7) R^1 = H, R^2 = imidazol-5-yl



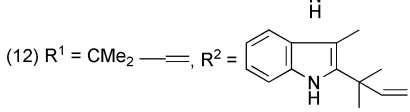
(4) R^2 = Ph
(5) R^2 = $CHMe_2$



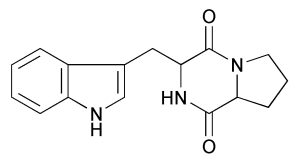
(10) R^1 = H, R^2 =



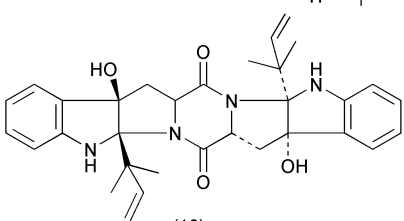
(11) R^1 = CMe_2 ═, R^2 =



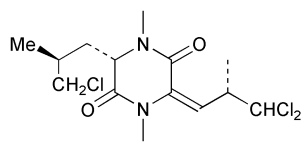
(12) R^1 = CMe_2 ═, R^2 =



(8)
(9) dehydro Pro analogue of (8)



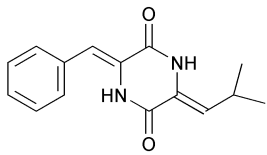
(13)



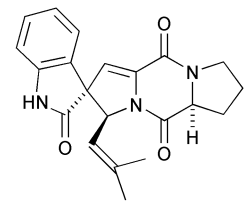
(14)

Biosynthetic experiments[16] have shown that condensation of tyrosine into its dioxopiperazine, followed by oxidation to its DOPA equivalent, represent steps to the ecteinascidins. Albonoursin (**15**) is biosynthesised[17] by dehydrogenation at the α,β-positions of cyclo(Phe-Leu), which appears to take place one side chain at a time. Cytotoxicity, however, is only shown when both dehydro positions have been created.

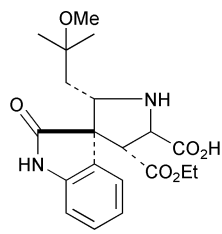
The promising antimitotic arrest properties of spirotryprostatin B (**16**) have initiated at least four separate syntheses. Following on from their synthesis of spirotryprostatin A (**16** + methoxy group in the indole ring) the Danishefsky group[18] have completed a rapid five-step synthesis of (**16**) in 7% overall yield. Key to the synthesis was a Mannich insertion reaction using a tryptophanyl ester, a proline derivative and 3-methylbut-2-enal as the three starting components. Insertion of a D-Pro ester into (**17**) was part of a nine-step strategy used by Williams *et al.*[19] A more biomimetic route was chosen by Ganesan *et al.*[20] whereby a tetrahydro precursor of (**16**) was synthesised and then oxidised by *N*-bromosuccinimide to form (**16**). In the fourth synthesis from Overman *et al.*,[21] the spiro indole rings were constructed from (**18**) using an asymmetric Heck cyclisation which yielded three stereoisomers.



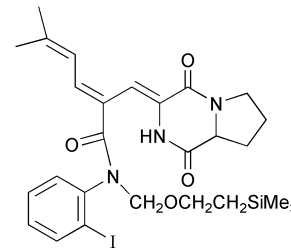
(15)



(16)



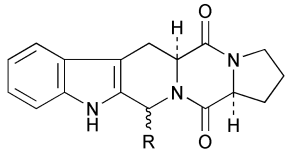
(17)



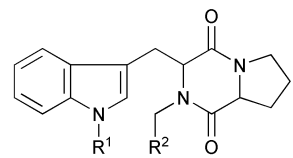
(18)

The cell cycle inhibition properties of the tryprostatins have been surveyed in the context of their promise as anti-cancer drugs. Their structures are based on the isoprenylation of the tryptophan moiety of cyclo(Trp-Pro). Thus several analogues such as (**19**) and (**20**) have been investigated.[22] Analogues of (**19**) with R^2 = 3,4,5-trimethoxyphenyl or phenylethenyl were more potent than the natural product, while the activity of analogues (**20**, R = Ph or 3,4,5-trimethoxyphenyl) depended on whether the R group was α or β on the carboline ring. In order to test their inhibition of microtubule assembly, analogues (**21**, **22**) have also been synthesised.[23] Analogue (**22**) inhibited cell proliferation at a concentration of 100 μM. The microtubule binding agents, phenylahistin (**23**) and aurantiamine (**24**) have been synthesised[24] from *N*,*N*-diacetyl derivatives of cyclo(Phe-Gly) and cyclo(Val-Gly) respectively, utilising a substituted imidazole as the other fragment for condensation.

A novel dioxopiperazine (**25**) which can function as a β-turn constraint has been synthesised.[25] The combination of an α,α-diamino arrangement and a β-keto ethoxycarbonyl was not conducive to easy cyclisation of linear precur-



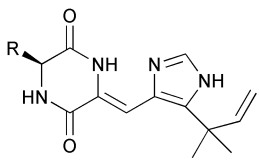
(20)



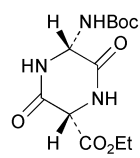
(19) R^1 = H, R^2 in script

(21) R^1 = H, R^2 = 

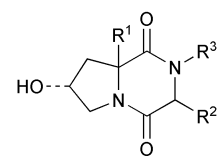
(22) R^1 = CH_2CH  , R^2 = 



(23) R = $PhCH_2$-
(24) R = i-Pr-

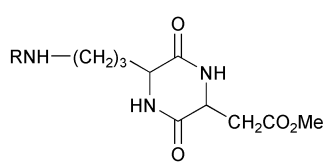


(25)

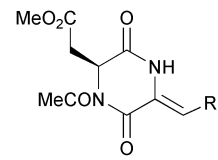


(26)

sors. General solid phase methods have been established[26] for the synthesis of hydroxyprolyl dioxopiperazines of the general structure (**26**). Linkage to the resin was made *via* the 4-hydroxy group of proline. In a related study[27] using magic angle spinning NMR spectroscopy to characterise the reaction steps, use of a polyethylene glycol resin gave a much better resolution of proton signals than was possible with polystyrene based resins. Synthesis of RGD mimetics (**28**) based on (**27**) have been reported.[28] Very little platelet aggregation inhibitory activity was found in these analogues. Penicillin acylase-catalysed condensation[29] of enantiomeric phenylglycines to form linear dipeptide methyl ester precursors has provided, after cyclisation in aqueous methanol, both cyclo (L-PheGly-L-PheGly) and cyclo(D-PheGly-L-PheGly).



(27) R = Z
(28) R = NH_2–C(=NH)NHX–CO
with X = -$CH_2C_6H_4$- or -C_6H_4-

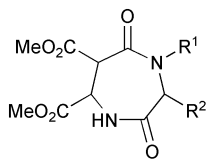


(29)

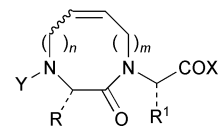
When dehydrodioxopiperazines (**29**) are hydrogenated[30] over 5% Pd/C, they undergo face-selective hydrogenation in excellent diastereoisomeric ratios of > 97%. Double-bond migration and *E*/*Z* isomerisation is experienced[31] by 3-alkylidene- or 3-benzylidene-2,5-piperazinediones in the presence of catalytic amounts of acid. Simple dioxopiperazines such as cyclo(Gly-Gly) and cyclo(Ala-Ala) assemble into supramolecular tapes[32] which can function as scaffolds to position co-crystallised guest molecules and can also cause physical gelation[33] in a wide variety of organic fluids, such as edible oils and glyceryl esters.

The molecular structure of dioxopiperazines has been re-visited. A new study[34] from the microwave spectral details of cyclo (Gly-Gly) reveals that the molecule in isolation does not adopt the planar structure deduced by X-ray crystallography, but is a boat conformation with C_2 symmetry. FT-IR Spectroscopy incorporating differential scanning calorimetry has been focused[35] on the intramolecular solid state cyclisation of H-Asp-Phe-OMe (the sweetener aspartame) to its dioxopiperazine. The initial step was loss of methanol, followed by a S_N1 attack of a nitrogen lone pair on an acyl cation.

Cyclic dipeptide nomenclature has to include a wider connotation than 2,5-dioxopiperazines, in that it is now possible to synthesise[36] seven-membered ring dipeptides of general structure (**30**) from linear precursors using the BOP reagent. Ring systems containing alkene bonds such as (**31**) have also been listed as cyclodipeptides and synthesised[37] by ring closure at the double bond using the Grubbs ruthenium catalyst.



(30)

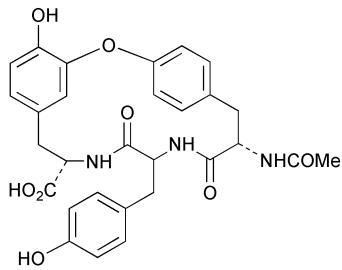


(31) n = 1 or 2, m = 1, 2 or 3

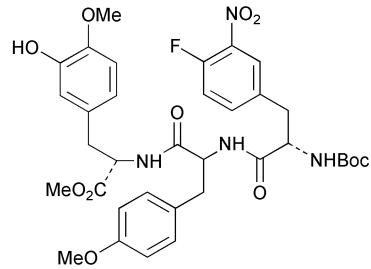
2.3 Cyclotripeptides. – Since homodetic cyclic tripeptides are seldom encountered, we can discuss under this sub-heading cyclic tripeptides with non-peptidic links making up the macrocycle. An important example of this is the non-competitive ACE-inhibitor K-13 (**32**). While total syntheses have previously been reported, two more ways of approaching the macrocyclisation to form K-13 have been reported. Quoted as one of the most efficient to date is the cyclisation[38] of (**33**) *via* an S_NAr to form the biaryl ether bond, while the macrocyclisation[39] of (**34**) utilised a palladium catalysed C–C bond formation.

2.4 Cyclotetrapeptides. – The structural elucidation of diheteropeptin (**35**), a TGF-β-like active substance from *Diheterospora chlamydosporia*, has been reported.[40] Apicidin (**36**) is a known inhibitor of histone deacetylase which regulates transcription and the assembly of newly synthesised chromatin. Studies have been reported[41] aimed at improving its selectivity by chemical modification. Ruthenium tetraoxide degradation of the tryptophan indole nucleus provided access to analogues with better selectivity than apicidin, while modifications at the keto side chain also produced more potent histone deacetylase inhibitors.

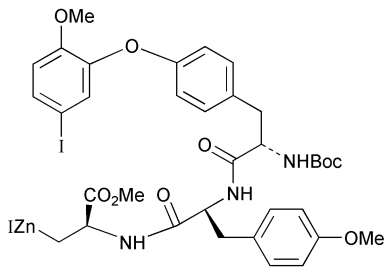
Although defined as a cyclic decapeptide, the kinin B_2 receptor antagonist, MEN 11270 (**37**) has a cyclotetrapeptide at its core. A solid phase synthesis[42] has been developed which assembles the linear sequence on-polymer, followed by a solution phase cyclisation at point (a) in the structure (**37**) utilising HATU. When EDC/HOBt was used for cyclisation there was a 31% racemisation of the argininyl residue. The synthesis of the all L-form of cyclo (Leu-Pro-Leu-Pro) is difficult because of the predominant formation of the cyclodimer during cyclisa-



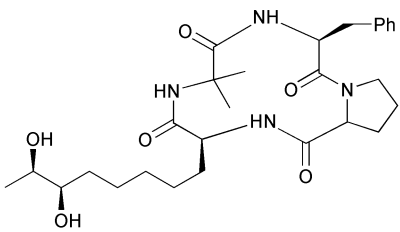
(32)



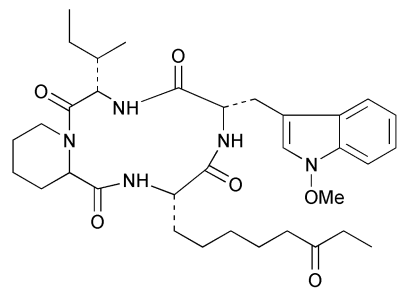
(33)



(34)



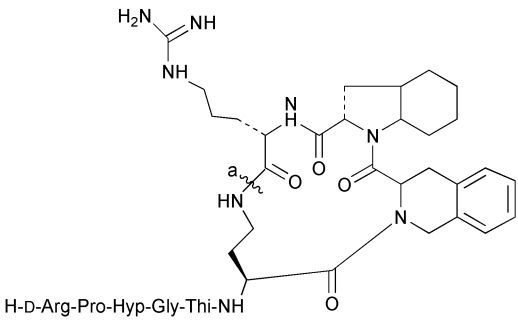
(35)



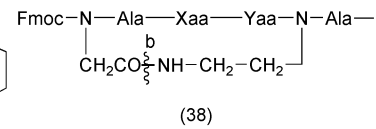
(36)

tion. However, it has now been reported[43] that using HATU under high dilution conditions the cyclic monomer becomes the predominant product. The backbone cyclisation kinetics [at point (b)] for compounds in the series (**38**), where Xaa and Yaa represent different combinations of Gly, Ala and Pro, has been investigated[44] experimentally and computationally. A cyclisation-prone conformation of the starting material was used to predict cyclisation tendency and this model was able to correctly rank cyclisation rates if they differed by 2-fold or more.

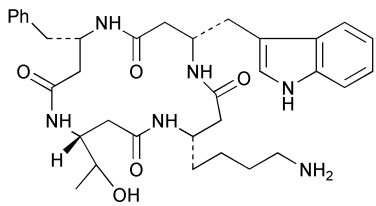
The development of cyclo β-tetrapeptides as mimics of natural peptide hormones has received a boost with the synthesis and conformational study[45] of cyclo (β-HPhe-β-HThr-β-HLys-β-HTrp) (**39**). The predicted conformation had to be modified in the light of NMR data, but a β-turn and equatorial positioning of the side chains still meant that (**39**) was a good mimic of somatostatin/



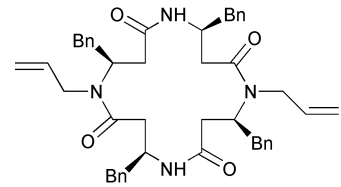
(37)



(38)



(39)

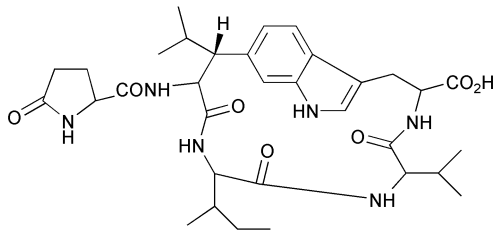


(40)

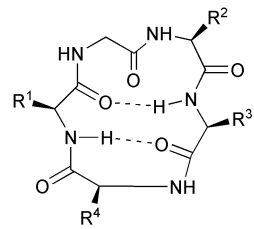
octreotride giving micromolar affinities with human somatostatin receptors, hsst1–5. A sulfonamide-based protection strategy[46] has made the cyclo β^3-tetrapeptide scaffold (**40**) available on a multi-gram scale.

2.5 Cyclopentapeptides. – A novel chitinase inhibitor, argifin, cyclo[(N^{ω}-*N*-methylcarbamoyl)-Arg-MePhe-β-Asp-β-Asp-D-Ala] has been discovered[47] in the culture broth of the fungus *Gliocladium* sp. FTD-0668. It has an IC_{50} value of 3.7 μM against blowfly chitinase. (6-Leucinyl-3′-yl) tryptophan is a novel feature[48] in the macrocycle of stephanoic acid (**41**) isolated from the stem of *Stephanotis floribunda*, making it a distant relative of the bicyclic toxic octapeptide, moroidin.

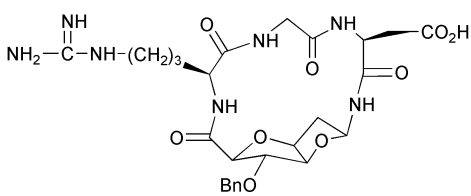
An improved and environmentally friendly synthesis[49] has given multi-gram quantities of cyclo(Arg-Gly-Asp-D-Phe-Lys), the highly potent and selective inhibitor of $\alpha_v\beta_3$ integrin. As compared with Kessler's original synthesis, this time the linear peptide was cyclised using 1-propane phosphonic acid cyclic anhydride and the Mtr protection of Arg was replaced by the more readily removed 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pbf) group. Another series of analogues[50] in the cyclic RGD pentapeptide series have been synthesised and investigated. Cell adhesion results in the analogue (**43**) in which γ-carboxy glutamic acid replaces Asp, proved positive, yet its congeners do not show activity in binding assays with recombinant integrin receptors. The β/γ-turn conformation shown in the structures (**42**) and (**43**) seemed to support the NMR data. It has been shown[51] that a cyclic RGD loop can be maintained by replacing two amino acid residues with bicyclic sugar azido acids as represented by (**44**). However, the universal acceptance of the β/γ-turn for cyclopentapeptides has been questioned[52] in a NMR/computational study based on a comparison



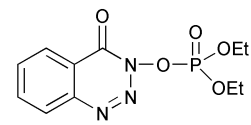
(41)



(42) R^1 = -$(CH_2)_3$NH-C(=NH)NH_2, R^2 = CH_2COOH, R^3 = R^4 = CH_2Ph
(43) R^1 = -$(CH_2)_3$NH–C(=NH)NH_2, R^2 = $CH_2CH(CO_2H)_2$, R^3 = CH_2OH, R^4 = $(CH_2)_4NH_2$



(44)



(45)

between results for cyclo(Pro-Ala-Ala-Ala-Ala), cyclo(D-Pro-Ala-Ala-Aib-Ala), cyclo(Arg-Gly-Asp-D-Phe-Val) and cyclo(Arg-Gly-Asp-Phe-D-Val). It is suggested that the βII′/γ- model is not valid if the γ-turn is centred at the amino acid residue of L-configuration. Bioactive cyclic RGD peptides of known conformations have also been used in a study[53] of the effect of inserting β-amino acid residues into the backbone. It is reported that insertion of the β-residues into cyclic tetra- and penta-peptides results in stabilisation of the secondary structure and these residues preferably occupy the central sequence of a γ-turn. *Ab initio* prediction[54] of the solution structures and populations of cyclopentapeptides in DMSO has been investigated using cyclo(D-Pro-Ala-Ala-Ala-Ala) as a model. Solvation parameters and the solvent accessible surface area of certain atoms were incorporated into the calculations.

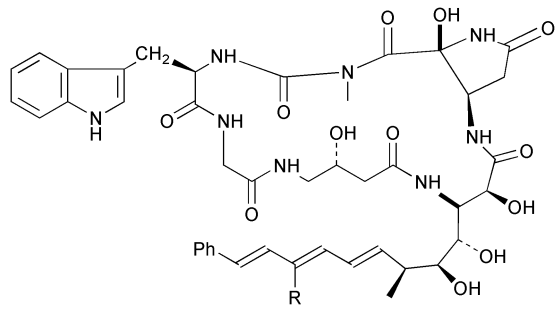
Two cyclopentapeptides, cyclo(Ala-Tyr-Leu-Ala-Gly) and cyclo(Pro-Tyr-Leu-Ala-Gly) from a Chinese medicinal herb have been synthesised[55] by activation of the Gly carboxyl group with the novel DEPBT reagent (**45**). The side chains of Ser and Tyr could remain unprotected and cyclisation yields of $> 50\%$ were realised. The first X-ray analysis[56] of an endothelin-1 antagonist has been

reported. The sodium salt of BQ 123 [cyclo(D-Trp-D-Asp$^-$-Pro-D-Val-Leu) Na^+] exists in four conformational isomeric forms and two Na^+ caged structures. Mass spectrometry has defined[57] the metal binding sites in BQ123, in an effort to clarify the ability of endothelin antagonists to induce net tubular sodium resorption in the proximal tubule cells and reverse acute renal failure. Collision activated decomposition techniques allied to an ion trap mass spectrometer have shown that for BQ123 and JKC301(an Ile analogue of BQ123) their Pro CO group is involved in alkali metal binding, although other ligands may be involved in the low energy structures of the complexes.

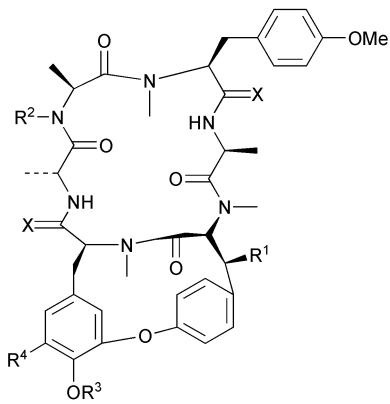
2.6 Cyclohexapeptides. – The antitumour/antifungal family of cyclic peptides, the microsclerodermins, is expanding, with the identification[58] of microsclerodermins F (**46**), G (**47**) and their dihydroindole analogues H and I, in deep water lithistid sponge *Microscleroderma* sp. Dehydromicrosclerodermins C and D have also been identified[59] in the marine sponge *Theonella cupola*. The roots of *Rubia cordifolia L.* have yielded[60] a novel antitumour dimer (**48**) of the well known RA-type structure in which two molecules of deoxybouvardin are linked together *via* an ether linkage. The significance of the cycloisodityrosine unit in RA-VII has been explored[61] *via* the degradation of its [N-MeAla^2]-RAVII analogue (**49**). Thionation of (**49**) to (**50**) using the Lawesson reagent, followed by methylation, hydrolysis and Edman degradation, gave the protected cycloisodityrosine unit (**51**).

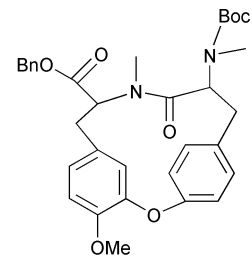
Through synthesis and conformational studies, structure–activity relationships have been worked out[62] for analogues of L-363,301 [cyclo(Phe-Pro-Phe-D-Trp-Lys-Thr)] and the cyclic octapeptide, octreotride. When the Pro residue was replaced with *N*-alkylated Gly residues and the residues both sides varied in turn, with Phe or Nal, the resulting analogues had increased selectivity towards the hsst 2 receptor. Potent cyclic peptide inhibitors of very late antigen-4 (VLA-4) have been discovered through analogue synthesis.[63] Within a series of cyclo hexa-, hepta-, and octa-peptides, cyclo(MePhe-Leu-Asp-Val-D-Arg-D-Arg) (ZD 7349) had a sufficiently promising spectrum of activity to undergo preclinical investigation. ZD 7349 inhibits MOLT-4 cell adhesion to fibronectin and vascular cell adhesion mol-1 with IC_{50} values of 260 and 330 nM respectively without showing any significant effect against other integrins. ($IC_{50} > 300\ \mu M$). It also inhibits ovalbumin-induced delayed type hypersensitivity (DTH) response in mice and is also active in type II collagen-induced arthritis and experimental auto-immune encephalomyelitis tests at doses of 3–10 mg kg^{-1}. The effects of structural modifications on the antifungal properties of echinocidin B (**52**) have been reported.[64] Several amino acids in zones A, B, and C have been replaced as well as the lipophilic side chain G. The best analogue (**53**) showed a five-fold improvement in efficacy as an antifungal reagent.

The synthesis[65] of cyclo(D-Trp-Gly-Pal-Pro-Gly-Phe) from its linear precursor activated as the Phe *p*-nitrophenyl ester can be catalysed by the antibody 16G3. There was a 22-fold enhancement in the rate of cyclisation giving the cyclohexapeptide in 90% yield. The appropriate stereochemistry of Trp and Phe was important for catalysis to take place. Cyclic hexapeptides with alternating



(46) R = H
(47) R = Me



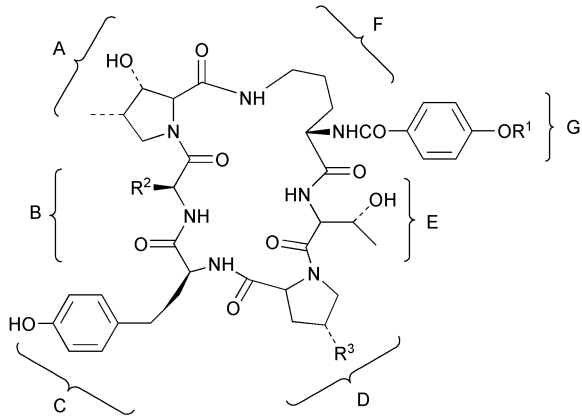


(51)

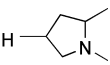
(48) R^1 = H, R^2 = H, X = O,
R^4 = another molecule of (48) linked as a dimer *via* R^3
(49) R^1 = H, R^2 = R^3 = Me, X = O
(50) R^1 = H, R^2 = R^3 = Me, X = S

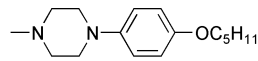
amino acids and 3-aminobenzoic acid residues, bind cations and anions with high affinity. It was perceived that anions link on to the amide NHs, so in order to reduce this interaction and hence strengthen cation affinity, methoxycarbonyl groups, as in (**54**), have been introduced[66] to lock the NHs by H-bonds. Both the linear tri- and hexa-peptide active esters have been used[67] for the synthesis of cyclo(Pro-Leu-Aib)$_2$. The more sterically-hindered Aib residue reduced the yields to around 18% and from NMR data *cis* and *trans* Aib-Pro bonds were shown to be present. Circular dichroism data revealed that a type II′ β-turn was present when Aib-Pro bonds were *trans*, and a type VI β-turn when they were *cis*.

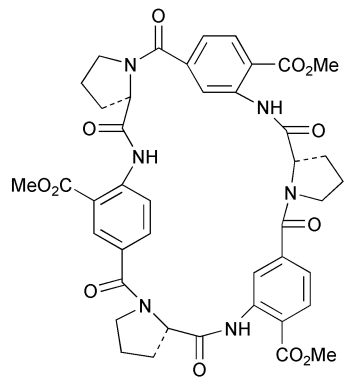
2.7 Cycloheptapeptides. – Mahafacyclin A from the latex of *Jatropha mahafalensis* has the structure[68] cyclo(Gly-Thr-Ile-Leu-Gly-Val-Phe). From NMR data it has been shown to have two β-turns at Gly^1-Thr^2 and Leu^4-Gly^5 and a H-bonding pattern involving Phe^{7}NH and Ile^{3}CO which is consistent with a β-bulge, often seen in cycloheptapeptides containing proline, but not seen before in the absence of Pro. Mahafacyclin A shows moderate antimalarial



(52) $R^1 = Me(CH_2)_7–$, $R^2 = MeCHOH$, $R^3 = OH$

(53) Zone A = $NH_2–C(=NH)NH$  , $R^2 = NH_2$, $R^3 = OH$

$R^1 =$ 



(54)

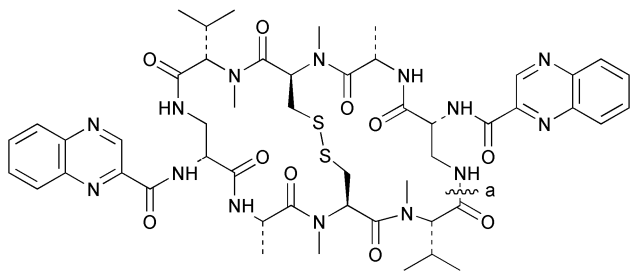
activity with IC_{50} value of 16 μM. Problems with the synthesis of both *R*- and *S*-Asn forms of phakellistatin 5 [cyclo(Phe-Asn-Ala-Met-Ala-Ile-Pro)] have been overcome[69] by a new solid phase strategy starting with Fmoc-Asp-α-OAllyl attached to the PAL resin *via* its β-COOH group. After assembly of the heptamers, and removal of the allyl group, cyclisation was carried out on-resin by PyAOP. The *R*-Asn analogue was obtained in 28% yield while its *S*-analogue was obtained in 15% yield. Chemical and physical properties of the *S*-Asn form were more in line with the natural form, yet the biological properties did not correspond, suggesting that the natural form is either contaminated with a trace of highly active cytotoxic material or there is a subtle conformational difference.

The known conformational data on axinastatin 2, cyclo(Asn-Pro-Phe-Val-Leu-Pro-Val), and axinastatin 3 with Val^4 replaced by Ile have been used to

assess[70] a statistical mechanical approach to predicting their conformation in DMSO solution. By incorporation of a solvation parameter with the GROMOS force-field energy functions, theoretical values for proton-proton distances and ^3J coupling constants agreed well with the NMR data.

2.8 Cyclooctapeptides/Cyclononapeptides. – Plants have become a rich source of cyclic peptides under this category. The whole plants of *Drymaria diandra* B1 are a source[71] of cyclo(Pro-Pro-Phe-Phe-Val-Ile-Ala-Phe-Leu) and drymarins A and B cyclo(Phe-Pro-Pro-Pro-Phe-Phe-Val-Ile-Ala) and cyclo(Pro-Phe-Tyr-Pro-Gly-Leu) respectively.[72] The roots of *Brachystemma calycinum* used for the treatment of rheumatism in folk medicine have yielded[73] brachystemin A, cyclo(Pro-Phe-Leu-Ala-Thr-Pro-Ala-Gly), while polycarponin A, cyclo(Pro-Gly-Phe-Phe-Ala-Ile-Ala-Ile-Pro), has been isolated[74] from *Polycarpon prostraltum*. Two cyclodipeptides as well as two new cyclooctapeptides, psammosilenin A and B with structures cyclo(Pro-Phe-Pro-Phe-Phe-Ala-Pro-Leu) and cyclo(Pro-Gly-Phe-Val-Pro-Phe-Thr-Ile) have been identified[75] in the roots of *Psammosilenin tunicoides*.

As a preparative study for eventual combinatorial synthesis, the analogue (**55**) of triostin A has been synthesised[76] in the solution phase. The disulfide link to complete the left hand side ring of (**55**) was first constructed, followed by cyclisation at position 'a' using EDCI/HOAt under high dilution. The quinoxaline groups were introduced as the last stage.



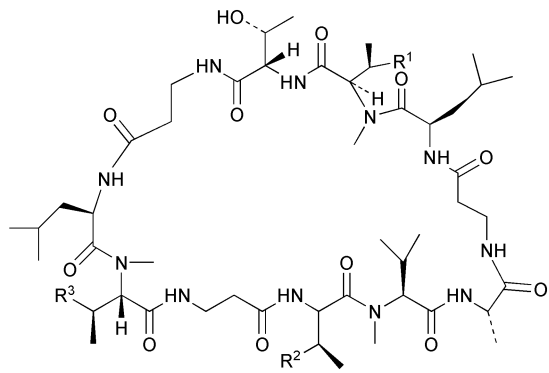
(55)

A solid state structural analysis[77] on cyclo(Pro-Pro-Phe-Phe-Ac_6c-Ile-D-Ala-Val), which contains the bulky $C^{\alpha\alpha}$-dialkylated 1-aminocyclohexane-1-carboxylic acid residue (Ac_6c) has been carried out. This cyclolinopeptide A deletion analogue shows a 'banana-twisted' conformation with a *cis* peptide bond between two Pro residues. A type VIa and two consecutive type III/I β-turns can be recognised, together with a γ-turn and a C_{16} bend. A conformational and ion-binding comparison has been made[78] on cyclic octapeptides containing reduced numbers of heterocyclic rings when compared with ascidiacyclamide 1 and patellamide D. Cyclo[Thr-D-Val-(Thz)-Ile]$_2$ with two thiazoles and cyclo(Thr-D-Val-αAbu-Ile)$_2$ with no five-membered rings were used. NMR data showed that the natural products' saddle conformation is retained in solution. Two thiazoles in the macrocycle pushed the conformation into a chair shape while no heterocyclic rings increased the conformations flexibility, with the main

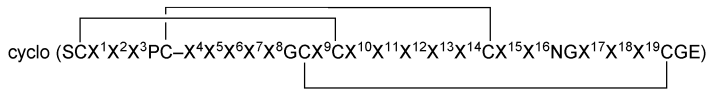
conformations being planar and stabilised by a series of transannular H-bonds. Decreasing the number of heterocyclic rings increased the affinity for 1:1 Ca^{2+} binding. Whether this is due to more flexibility in the ring or the increased number of CO groups for binding is not clarified. With the aim of mimicking cyclodextrin's capacity to act as adapters for the pore-forming protein, staphylococcal α-homolysine (αHL), cyclooctapeptides have been examined.[79] Four examples, cyclo(Arg-D-Leu)$_4$, cyclo(Glu-D-Leu)$_4$, cyclo[Phe-D-*N*(aminomethyl)Ala-Phe-D-Ala]$_2$ and cyclo[Phe-D-*N*(carboxymethyl)Ala-Phe-D-Ala]$_2$ became lodged in the αHL pore, altering its unitary conductance and ion selectivity. The positively charged cyclooctapeptides were also able to bind polyanions.

2.9 Cyclodecapeptides and Higher Cyclic Peptides. – The *Theonella swinhoe* sponge continues to be a rich source of diverse cyclic entities. The previously identified cyclic undecapeptide barangamide A (**56**) from this sponge has been augmented by the identification[80] of barangamides B–D with structures (**57–59**) respectively. The tropical plant *Leonia cymosa* has yielded[81] four novel anti-HIV macrocyclic peptides, cycloviolins A–D (**60–63**). MALDI-TOF and FAB MS, combined with enzymic digestion, secured evidence for the three intramolecular disulfide bridges and the structures show similarities to cyclopsychotride A and circulins A and B. Another tropical tree, *Chassalia parvifolia*, produces macrocyclic peptides which augment the circulin family to include circulins C–F which have been shown[82] to have the structures (**64–67**).

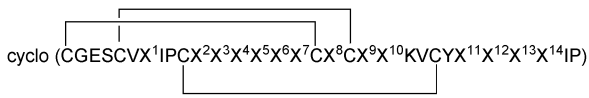
Preparative scale syntheses[83] of NEtXaa analogues at position 4 of cyclosporin A (**68**) have been reported. The route involved a four-step opening of the parent structure (**68**) at position 4, and then incorporating the Xaa residues in turn (Xaa representing Leu, Val, Ile and Thr). The linear undecapeptide precursor was cyclised in each case between residues 4 and 5 using Carpino's TFFH reagent. The majority of the analogues showed comparable binding affinities to cyclosporin A (**68**), but strongly reduced immunosuppressive activity. A 4 + 7 fragment condensation has yielded[84] the linear undecapeptide necessary to form



(56) R^1 = Et, R^2 = Et, R^3 = Et
(57) R^1 = Et, R^2 = Et, R^3 = Me
(58) R^1 = Et, R^2 = Me, R^3 = Et
(59) R^1 = Me, R^2 = Et, R^3 = Et



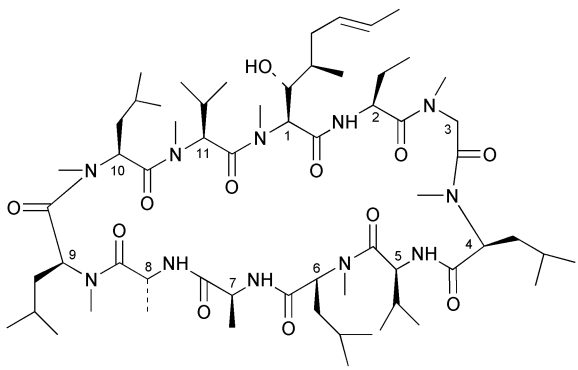
	X^1	X^2	X^3	X^4	X^5	X^6	X^7	X^8	X^9	X^{10}	X^{11}	X^{12}	X^{13}	X^{14}	X^{15}	X^{16}	X^{17}	X^{18}	X^{19}
(60)	V	F	I	I	S	A	A	I	S	K	–	N	K	V	Y	R	V	I	P
(61)	Y	V	L	F	T	–	V	–	T	T	S	S	Q	–	F	K	T	A	–
(62)	V	F	I	L	T	T	V	A	S	K	–	N	K	V	Y	R	–	I	P
(63)	V	F	I	I	S	A	A	I	S	K	–	N	K	V	Y	R	–	F	P



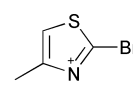
	X^1	X^2	X^3	X^4	X^5	X^6	X^7	X^8	X^9	X^{10}	X^{11}	X^{12}	X^{13}	X^{14}
(64)	F	I	T	S	V	A	G	S	K	S	R	N	G	–
(65)	W	V	T	S	I	F	N	K	E	N	H	D	–	K
(66)	W	L	T	S	V	F	N	K	E	N	H	D	–	K
(67)	W	I	S	A	A	I	G	S	K	N	R	–	–	A

cyclosporin O, ([MeLeu[1]] cyclosporin A). Linear peptides were constructed in solution and the solid phase using novel thiazolium (BEMT) or pyridinium (BEP) reagents having structures (**69**) and (**70**) respectively. Cyclisation between the two alanine units was effected by HAPyU /DIEA in yields ranging between 70 and 84%.

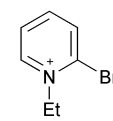
Acid catalysed degradation of cyclosporin A (**68**) has also produced material for an X-ray determination[85] of cyclosporin H([D-MeVal[11]]-cyclosporin A) as its diethyl ether–water crystalline complex. Two isomorphous clathrates formed by dihydrocyclosporin A (**68**) and cyclosporin V ([Abu[7]]-cyclosporin A) with t-butyl methyl ether have been studied[86] by X-ray crystallography. Using the



(68)



(69)

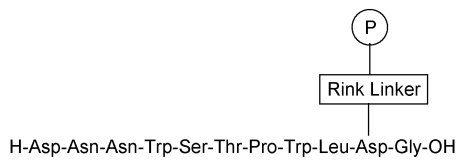


(70)

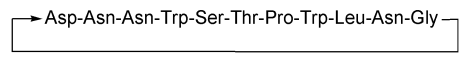
same technique, isomorphous clathrates involving cyclosporin A with THF or di-butyl ether have been proved[87] to have $P2_1$ symmetry. Both cyclosporin A and the *N*-hydroxyethyl derivative of [D-Ser] cyclosporin A (IMM-125), when reacted[88] with a cytochrome P 450-dependent mono-oxygenase system, show bio-

transformations at residues 4, 9 and 1. Non-enzymic formation of a THF derivative could be identified involving the side chain at position 1. None of the new derivatives showed immunosuppresive activity. A method[89] for the quantitative analysis of cyclosporin in blood has been developed using MALDI-TOF and SIMS-TOF mass spectrometry techniques.

The antibacterial undecapeptide kawaguchipeptin B (**72**) has been subject to a survey[90] of eleven different polystyrene resins for its on-resin synthesis, starting with the side chain attachment of an aspartyl residue on to a Rink resin. Having arrived at resin-bound (**71**) the cyclisation was then carried out using DIC/HOBt, which led to the conclusion that 3–6% DVB cross-linked polystyrene resin gave optimal performance. The side chain attachment of an Asp residue to the resin also initiated the on-resin synthesis[91] of the 29-mer cyclic antibacterial peptide (**73**) now undergoing pharmaceutical development as an analogue of rhocoricin. Instead of the usual protection of the Asp α-carboxyl by an allyl group, the Dmab (4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino} benzyl ester) was used to advantage, with a cyclisation yield of 20% being obtained using PyBOP/HOBt. A bicyclic undecapeptide (**74**) designed to mimic the Ca coordination site of calmodulin has been synthesised.[92] NMR Analysis in d_6-DMSO and in TFE/D_2O showed the presence of one stable conformer, exhibiting a *trans* conformation at both prolines, giving a rectangular shape made up of two anti-parallel β-strands connected by two β-turns. CD Spectra pointed to a 1:1 Ca^{2+}-peptide complex, with an association constant of *ca* $1 \times 10^5\,M^{-1}$.



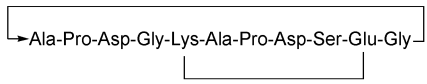
(71)



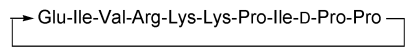
(72)

Small libraries of conformationally defined β-hairpin protein epitope mimetics based on Loop III of human-platelet-derived growth factor B have been synthesised.[93] The libraries were created from the templates (**75**) and (**76**). Two synthetic approaches were used, either Fmoc chemistry starting at a C-terminal Arg residue, followed by cyclisation using HATU/HOAt in solution, or macrocyclisation on-resin with the same reagents starting from Fmoc-Lys-*O*-Allyl first-coupled to chlorotrityl resin. Cyclisation in solution gave a cleaner product. A 14-residue cyclic gramicidin S analogue (**77**) has been investigated[94] to attain antimicrobial and hemolytic profiles. NMR methods confirmed a highly amphipathic anti-parallel β-sheet conformation, which could be changed by enantiomeric substitution. A direct correlation between high amphipathic character

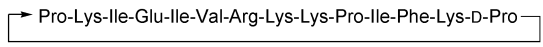
(73)



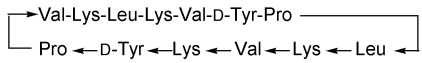
(74)



(75)



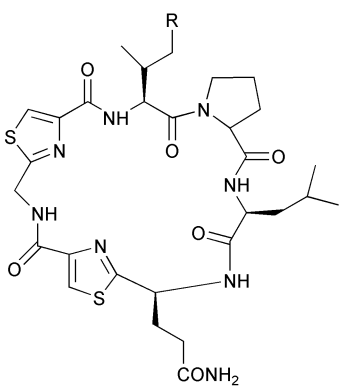
(76)



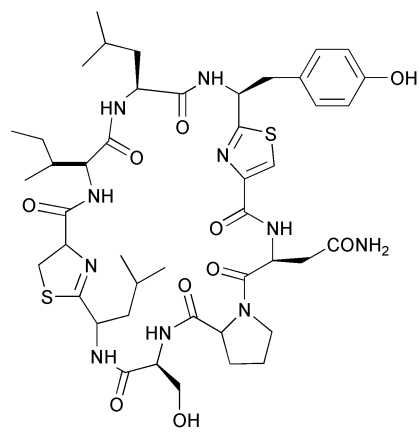
(77)

and potent hemolytic activity was found, whereas an inverse correlation existed between amphipathicity and antimicrobial function. A new synthesis[95] has been published for the C_{28}–C_{34} segment of FK 506, starting from the chiral building block, 6,8-dioxabicyclo[3.2.1]octane framework.

2.10 Peptides Containing Thiazole/Oxazole Rings. – The marine cyanobacterium *Lyngbya majuscula* has been investigated by two separate groups of researchers. In collections from Palau, as well as the known dolastatin 3 (**78**), novel compounds (**79**) and (**80**) have been identified.[96] From a Guamanian strain, lyngbyabellin A (**81**) has been isolated[97] which is a cyclodepsipeptide structurally related to dolabellin and mirabiamide E. The cytotoxic aqueous extract from the

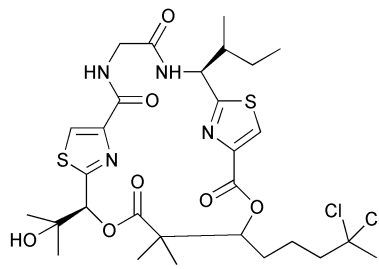


(78) R = H
(79) R = Me

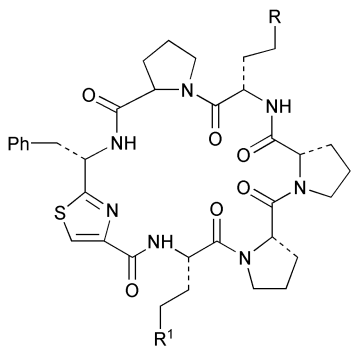


(80)

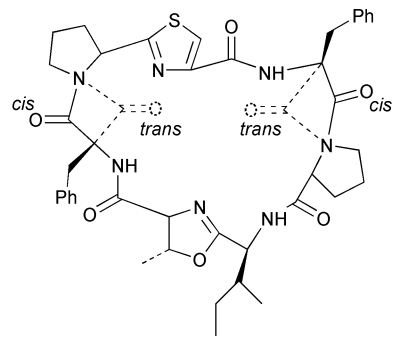
marine sponge *Haliclona nigra* has provided[98] two new cyclic hexapeptides, haligramides A (**82**) and B (**83**) as well as the known waiakeamide. Two isomers, *cis–cis* and *trans–trans* forms of ceratospongamide (**84**), have been identified[99] from the Indonesian red alga, *Ceratodictyon spongiosum* and its symbiotic sponge *Sigmadocia symbiotica*. The *cis–cis* form of (**84**) has a puckered conformation and is inactive, while the *trans–trans* form is planar and is a potent inhibitor of $sPLA_2$ expression in a model for anti-inflammation (ED_{50} 32 nM). Microcyclamide (**85**) has been hailed[100] as the first example of a cyclic hexapeptide from *Microcystis aeruginosa*. Its structure bears similarities to nostocyclamide, dendroamides, raocyclamides and the tenucyclamides but it was only moderately cytotoxic against P388 murine leukemia cells. Further examples of lissoclinamides have been isolated[101] from the ascidian *Lissoclinum patella*. Mass spectrometric techniques and NOE restrained molecular dynamics suggest that the lissoclinamides 9 and 10 have the structures (**86**) and (**87**) respectively.



(81)

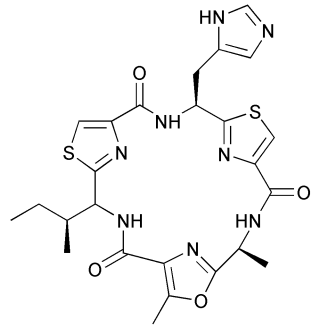


(82) R = R^1 = SMe
(83) R = SMe, R^1 = SOMe

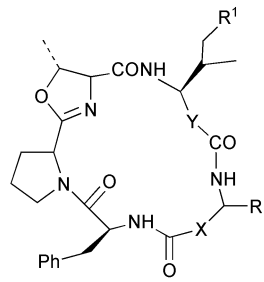


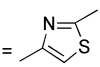
(84)

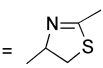
The total synthesis[102] of lissoclinamides 4 (**88**) and 5 (**89**) from *Lissoclinum patella* has highlighted the necessity to re-assign the configuration from the original designation. Precursors such as (**90**) show that the oxazoline and thiazoline rings can be assembled in a double cyclodehydration reaction from thioamide/amide precursors using the Burgess reagent. Macrocyclisation to form (**90**)

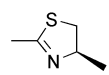


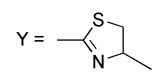
(85)

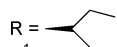


(86) X = 

(87) X = 

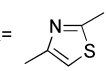
Y = 

Y = 

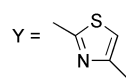
R = 
R^1 = Me

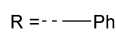
R = 
R^1 = Me

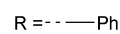
(88) X = 

(89) X= 

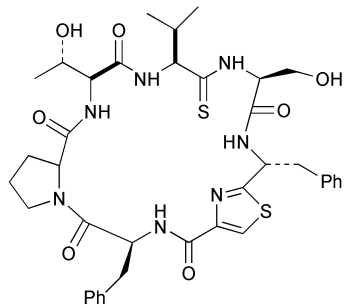
Y = 

Y = 

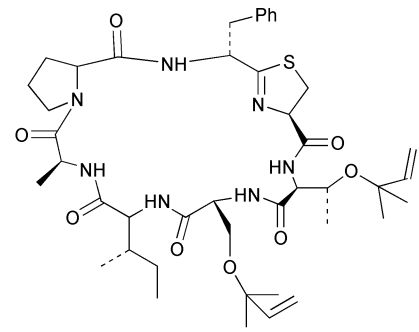
R = 
R^1 = H

R = 
R^1 = H

was carried out by DPPA/NMM in DMF. A total revision of stereochemistry, now incorporating D-Phe, was also required to confirm by total synthesis[103] the structure of trunkamide A (**91**), from the *Lissoclinum* sp. The authors' acid-assisted aziridine opening technique was used for the novel reverse prenylated Ser and Thr side chains as well as efficient oxazoline–thiazoline interconversion in the macrocyclic skeleton.

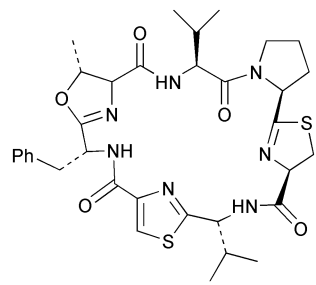


(90)

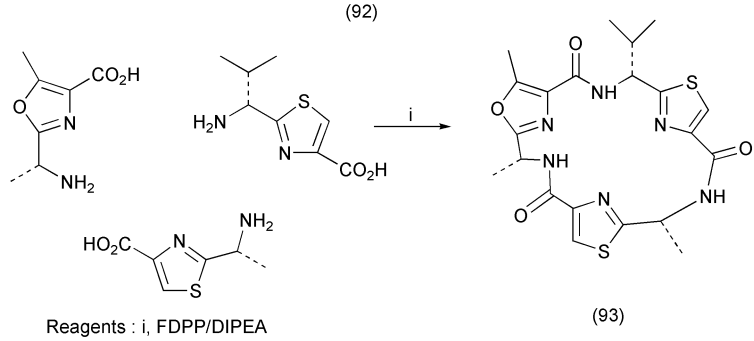


(91)

A similar synthetic strategy to the one reported above for the lissoclinums has also been used[104] for the synthesis of cyclodidemnamide (**92**) from the sea-squirt *Didemnum molle*. A reassignment of one of the Val units to the D-configuration had to be made before the spectroscopic data correlated. Alkali metal ions have been found to influence[105] the cyclooligomerisation of chiral thiazole- and oxazole-based amino acids to form cyclic trimers and tetramers. The scope of the reaction has been extended to reactions involving more than one heterocyclic amino acid, as summarised in Scheme 1, which gave a 23% yield of dendroamide A (**93**). Homo-coupled products were also produced in the reaction mixture. Cyclooligomerisation from single oxazole amino acids in a 'one-pot' procedure[106] has been used to synthesise functionalised platforms, such as (**94**), faster than by linear trimer assembly followed by cyclisation. The platform (**95**), based on the structure of the dolastatins, was however synthesised[107] *via* its linear precursor, which was cyclised using PyBOP in 86% yield.

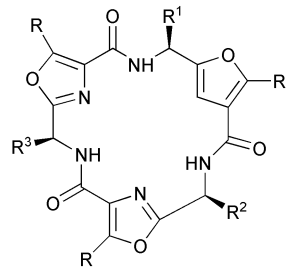


(92)

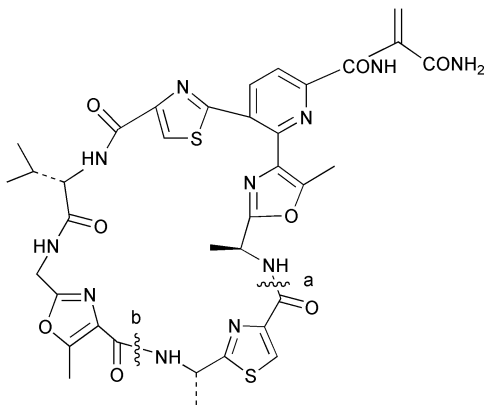


(93)

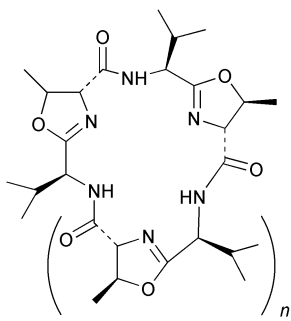
Scheme 1



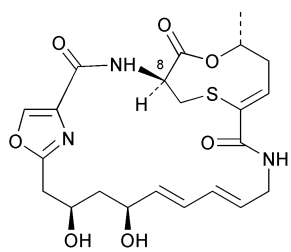
(94) R = H, R^1 = $(CH_2)_4NHZ$, R^2 = CH_2OBzl, R^3 = Me
(95) R = Me, R^1 = R^2 = R^3 = CO_2H



(96)



(97)

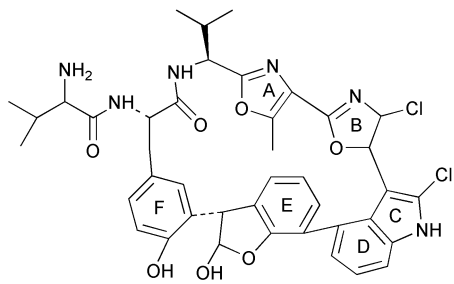


(98)

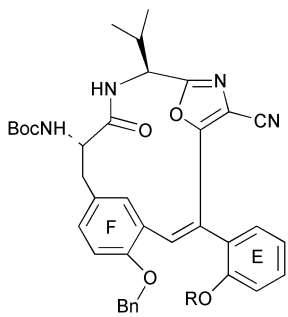
Two strategies[108] for cyclisation using pentafluorophenyl esters of linear precursors, to macrocyclise at either points (a) or (b) have successfully given the antibiotic promothiocin A (**96**). A modified Bohlmann-Rahtz pyridine synthesis provided the oxazolyl-thiazolyl-pyridine centrepiece, while dirhodium(II)-catalysed carbenoid N–H insertion was involved in making the oxazole building

blocks. The thiazoles were produced *via* the Hantzsch reaction, while the dehydroalanine unit was introduced at the end. Cyclooligomerisation of oxazoline amino acids takes precedent over dioxopiperazine formation. In a study[109] based on the units (**97**), the relative ring size ($n = 0$, 1 or 2) produced from single oxazoline precursors is determined by kinetic control and dependent on the relative stereochemistry at the α-carbons. Griseoviridin (**98**), a member of the streptogramin family of antibiotics, currently licensed in the USA against bacteria resistant to vancomycin, has been synthesised[110] for the first time after two decades of endeavour by many groups. The oxazole carboxamide link was chosen as the site for macrocyclisation, using EDCI/HOBt, and the epimer at C-8 was also obtained by using L- instead of the D-Cys unit.

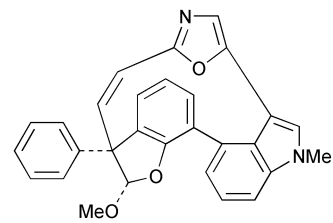
Competition is pretty keen in the attempts to synthesise the anti cancer agent diazonamide A (**99**) from the ascidian *Diazona chinensis*. In a three part report[111] intermediate fragments relating to rings A/B, C/D and E/F have been separately constructed. The triaryl ethylene component (**100**) has been prepared[112] as a latent equivalent of an aldehyde, which on hydroxylation of the alkene, the resulting pinacol undergoes a stereo-controlled rearrangement to an aldehyde for construction of the ring E/F link. Seventeen synthetic steps[113] were required to achieve the atropisomerically pure BCDEF core macrocycle (**101**). The bridge double bond was constructed using a Horner–Wadsworth–Emmons reaction while a Suzuki coupling gave the C_{16}–C_{18} biaryl linkage.



(99)

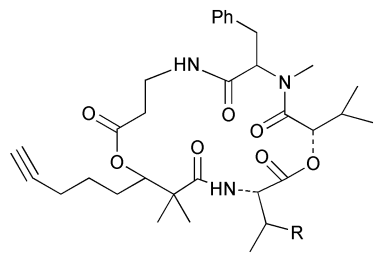


(100)

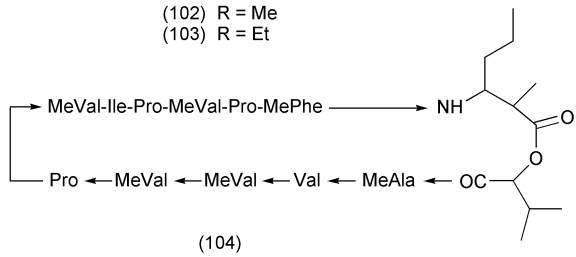


(101)

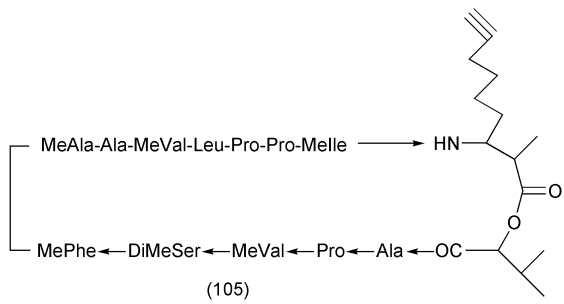
2.11 Cyclodepsipeptides. – Nature once more has proved to be a rich source of diverse structures under this category. As the first few examples show, the marine environment continues to be a productive source. Thus the marine cyanobacteria *Lyngbya majuscula* and *Schizothrix* sp. have yielded[114] from their lipid extracts yanucamides A (**102**) and B (**103**). *Symploca laete-viridis* has been shown[115] to contain at least two cyclodepsipeptides, malevamide B (**104**) and C (**105**), but neither possessed cytotoxicity. Tamandarins A (**106**) and B (**107**) from an ascidian of the *Didemnidae* family have been shown[116] to be very similar to didemnin B differing only in that the tamandarins have a hydroxyvaleric acid residue in them. They are three times more potent than didemnin B in the same cytotoxicity tests. The sponge *Theonella swinhoe* has yielded[80] yet another cyclodepsipeptide in the form of theonellapeptolide II_e (**108**). A toxic strain 97 of the cyanobacteria *Oscillatoria agardhii* has given rise[117] to oscillapeptilide 97-A (**109**) and 97-B (**110**), and a novel cyclononapeptide, oscillacyclin, while no such peptides were found in the non-toxic strains 2 and 18. From the latter, microviridin I (**111**) has been identified. Kahalide O (**112**) has been isolated[118] from the sacoglassan mollusk *Elysia ornata* and its algal diet *Bryopsis* sp., but showed no activity in contrast to its relative kahalalide F.



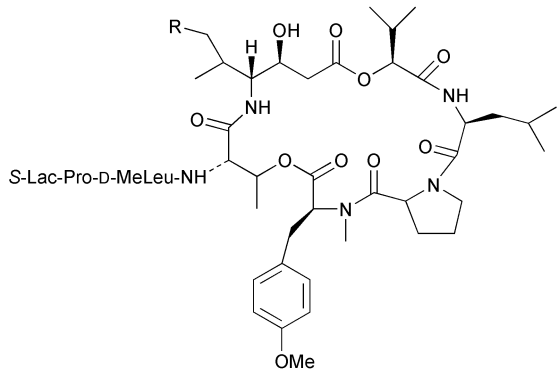
(102) R = Me
(103) R = Et



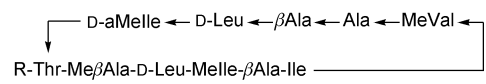
(104)



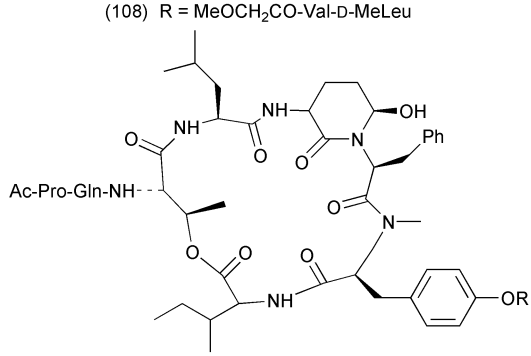
(105)



(106) R = Me
(107) R = H



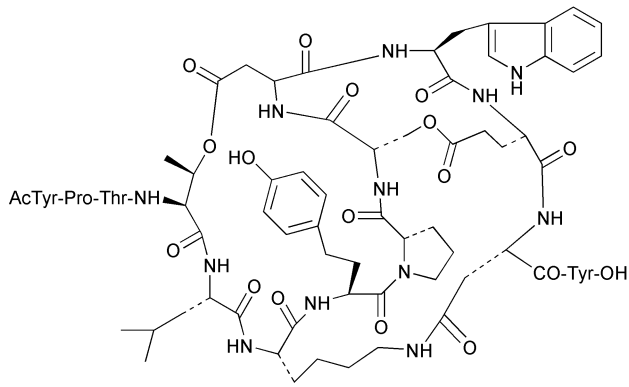
(108) R = $MeOCH_2CO$-Val-D-MeLeu



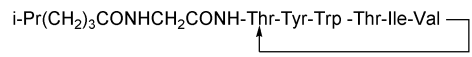
(109) R = Me
(110) R = H

Membership of the rakicidin family has been augmented[119] with a non-cytotoxic member, rakicidin C (**113**), found in a *Streptomyces* sp., and the same organism is also the source[120] of chloptosin (**114**), which is related to himastatin. Chloptosin produces apoptosis in apoptosis-resistant human pancreatic adenocarcinoma cell lines and showed strong antimicrobial activity against Gram-positive bacteria including MRSA. Four new lipopeptides with anti-fungal activity have been identified[121] in *Bacillus thuringiensis*. Named the kurstakins, they have been shown to have structures (**115–118**). Selective NK_2 antagonist activity (IC_{50} 68 nM) has been found in Sch 218157 (**119**) characterised[122] from a fungal fermentation bath, while glomosporin, an anti-fungal antibiotic from *Glomospora* sp. BAUA 2825 has been identified[123] as (**120**).

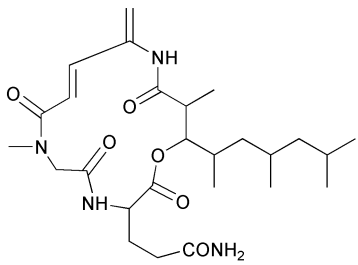
A short review[124] on cyclodepsipeptides has concentrated on the development of anthelmintically active synthetic enniatins. With the development of two efficient protocols for the synthesis of the major 'northern' component of crypto-



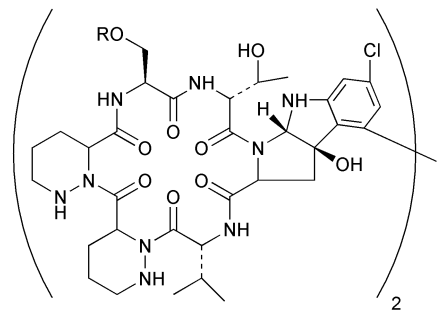
(111)



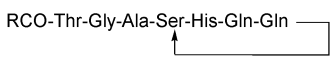
(112)



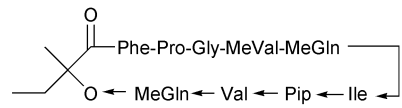
(113)



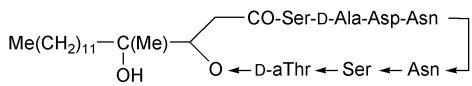
(114) In one segment R = Me and R = Ac in the other



(115) R = $Me_2CH(CH_2)_7$-
(116) R = $Me(CH_2)_{10}$-
(117) R = $Me_2CH(CH_2)_8$-
(118) R = $Me_2CH(CH_2)_9$-

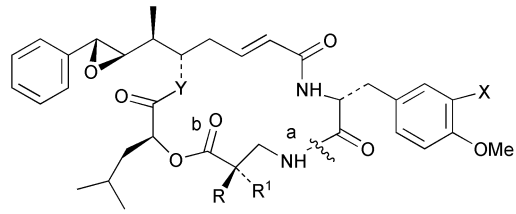


(119)

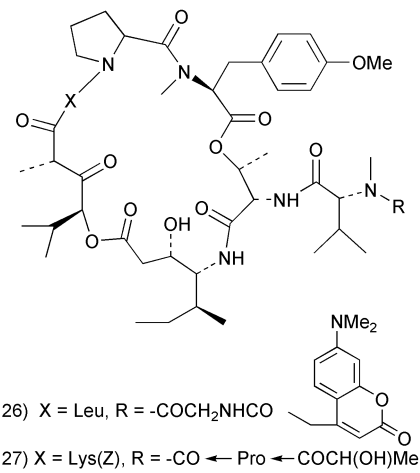


(120)

phycin-24 (arenastatin A) (**121**), full details for the total synthesis have now appeared.[125] A convergent synthesis[126] of (+)-cryptophycin B (**122**) from the cyanobacteria *Nostoc* sp. utilised a strategy for building up a linear precursor which facilitated macrocyclisation at position 'a' in (**122**), using 2,4,6-trich-



(121) R = R^1 = X = H, Y = O
(122) R = Me, X = H, R^1 = H Y = O
(123) R = Me, X = Cl, R^1 = H, Y = NH
(124) R = Me, X = H, R^1 = H, Y = O
(═ instead of epoxide)
(125) R = R^1 = Me, X = Cl, Y = O

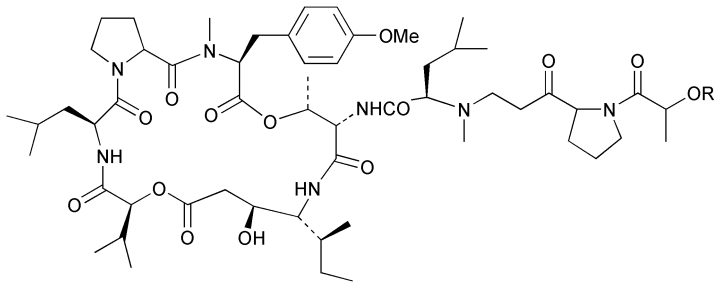


(126) X = Leu, R = -$COCH_2NHCO$
(127) X = Lys(Z), R = -CO ← Pro ← COCH(OH)Me

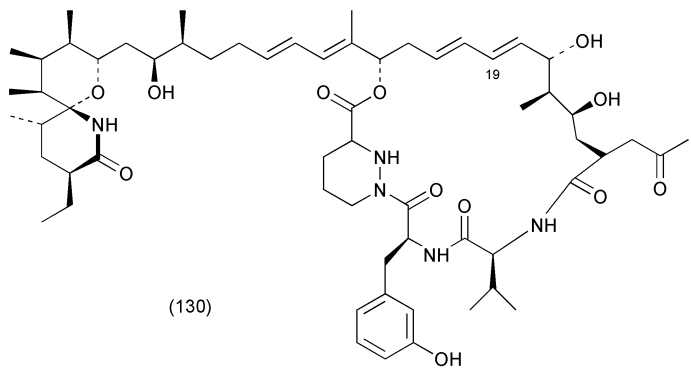
lorobenzoyl chloride/DMAP. Three analogues of arenastatin A (**121**) have been synthesised,[127] in which the two depside links in turn have been replaced by amide groups. Stability in serum is increased whenever the ester 'b' in (**121**) has been replaced by an amide, giving support to the observation that this ester is the group prone to metabolic breakdown. An amide at this position does not lead to loss of activity. Further modifications to the ester 'b' in (**121**) have included[128] a carba-analogue and a 20-deoxo analogue and both show good stability in serum and retain moderate cytotoxicity. A total synthesis[129] of cryptophycin 337 (**123**), the aza-analogue of cryptophycin 1 has revealed that the compound tends to undergo ready skeletal rearrangement. The chiral centre necessary to secure the correct stereochemistry of the styrene oxide group was derived from mandelic acid. Cryptophycin 4 (**124**), which lacks the epoxide ring, has been synthesised[130] using a chiral molybdenum cationic complex to secure the necessary configuration in the substitiuted phenyl octadienoic acid fragment. A Sharpless asymmetric dihydroxylation strategy has been used[131] to secure stereochemical control in epoxide formation during the synthesis of cryptophycin 52 (**125**).

A bioluminescent dimethylaminocoumarin probe has been attached[132] *via*

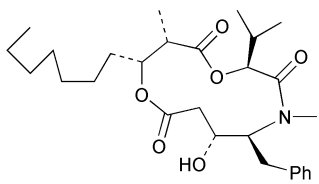
glycine to give the fluorophoric didemnin (**126**). The synthesis[133] of [*Z*-Lys^3]-didemnin B (**127**) has shown that the biological activity is retained and augers well for the potential linking of additional affinity ligands to the lysine side chain. The simplified tamandarin type macrocycle and the side chain of didemnin M have been combined[134] in the structure named [(2*S*)-Hiv^2] didemnin M (**128**), which has been shown to have comparable anti-tumour activity to didemnin B. Tamandarin B (**129**), which is [Nst^1, 2*S* Hiv^2] didemnin B has been synthesised[135] using a core tetrapeptide available from the authors' previously published synthesis of didemnins and coupling it with Hiv-norstatine. Macrocyclisation was achieved by HATU, followed by the attachment of the side chain using the DEPBT coupling agent.



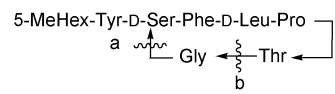
(128) R = -CO ←Gln←pGlu
(129) R = H



(130)



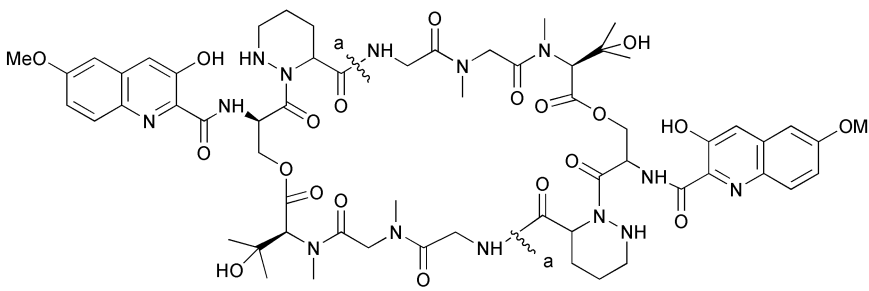
(131)



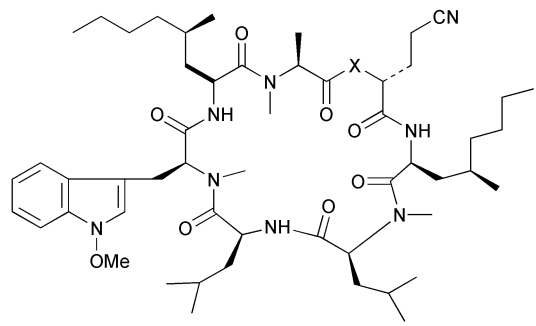
(132)

Full details have now been reported,[136] to support last year's preliminary communication, on the total synthesis of the immunosuppressant sanglifehrin A (**130**). A stereoselective synthesis[137] of the C_{13}-C_{19} fragment in (**130**) has also been accomplished. Pseudomycin B when used as an antifungal agent produces

irritation at the injection site. Although this can be removed by use of synthesised[138] 8-amido derivatives, the analogues showed less *in vivo* efficacy. Interest in hapalosin (**131**) continues with a further example of a total synthesis.[139] Macrocyclisation was at the amide bond using Shioiri's DPPA coupling agent. When an attempt was made to replace the hydroxyisovaleric residue by valine in order to make an amide analogue, a Curtius rearrangement of an acyl azide occurred, to give two isomeric ring-expanded analogues. In all three compounds synthesised in this report, NMR data confirmed a s-*transoid* rotamer at the tertiary amide bond. Kahalalide B (**132**) from the mollusk *Elysia rufescens* has been synthesised[140] from two different strategies with different points of cyclisation. Both linear precursors were assembled on 2-chlorotrityl chloride resin. The attempt to cyclise at the ester bond 'a' in (**132**) using PyBOP/DIEA gave a 28% yield, while cyclisation at 'b' only gave 22% yield, yet the practitioners favoured the latter strategy.



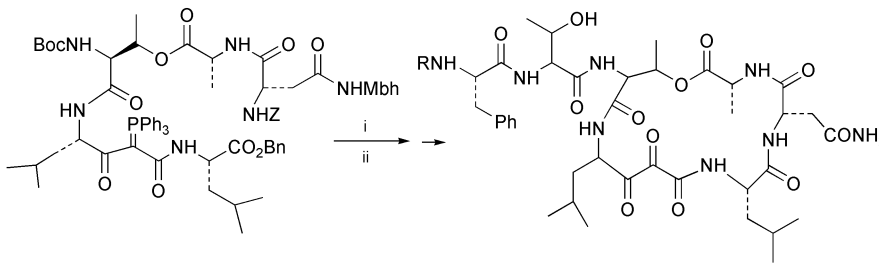
(133)



(134) X = O
(135) X = NH

A short review[141] on the authors' research into the reverse transcriptase inhibitor luzopeptin E2 (**133**) has appeared and the same authors have also reported[142] a total synthesis of (**133**). The target strategy was to cyclodimerise from a pentamer precursor and this was eventually achieved at points 'a' in (**133**) using EDCI/HOAt, which gave 26% of the cyclodimer and 10% of the cyclomonomer. The aza analogue (**135**) of HUN-7293 (**134**) has been synthesised,[143] together with its C_2^3 MeLeu epimer using solution phase techniques. Best results

in the cyclisation between MeLeu[3] and Leu[4] was obtained using the BOP reagent, and this strategy proved superior to one involving EDCI/HOAt at the CO–X position in (**135**). Both analogues were less active than the parent cyclodepsipeptide. Elastase inhibitors, YM-47141 (**136**) and YM-47142 (**137**) with the intriguing vicinal tricarbonyl system in their backbone, have been synthesised.[144] The later stages of the synthesis are represented in Scheme 2 and show how the phosphoranylidene group originally involved in activation is also a useful protecting group until it is unmasked at the final stage by oxidative cleavage of the C = P bond.



Reagents: i, Pd/C/H_2; ii, DPPA/$NaHCO_3$

(136) R = -$COCH_2Ph$
(137) R = -$COCH_2CHMe_2$

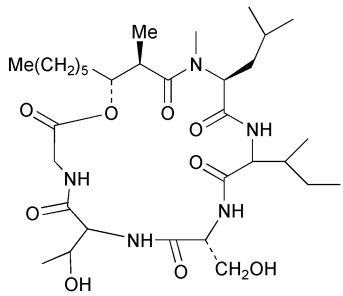
Scheme 2

Ambiguity in the absolute configuration at certain centres in globomycin (**138**) has been resolved[145] by an X-ray crystallographic analysis and a total synthesis. The linear precursor required for macrocyclisation at the Ser-aThr bond was constructed from three main fragments and cyclised in 45% yield using HATU. The azirine/oxazolone method for incorporating the hindered Aib residues into peptides has been put to good use in the synthesis of linear precursors to the cyclodepsipeptides (**139**)[146] and (**140**).[147] Presumably the predefined conformation of the linear precursor made it possible to cyclise in good yield at the depside link by heating in HCl/toluene at 100 °C.

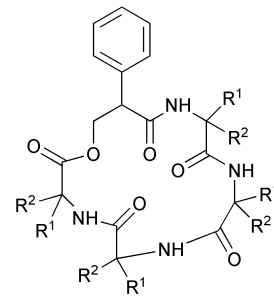
The configuration/conformation of FR 134043 (**141**), an inhibitor of human leukocyte elastase, has been studied[148] by NMR. A simulated annealing protocol was applied to 8 possible stereoisomers and the one that best satisfied the NOE distance constraints was chosen as the most representative structure. All the amino acid residues were found to be in the L-configuration with the seven side chains located outside the bicyclic framework.

3 Modified and Conjugated Peptides

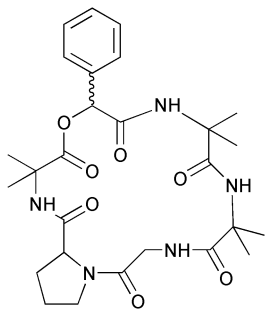
This section has been traditionally reserved for peptides that are often the result of post-translational modifications and therefore bear non-peptidic conjugate groups that are needed to maintain biological activity. The importance of these modifications has increased over the years, and as the conjugates are often linked *via* easily-metabolised and chemically fragile bonds, they demand special care in their synthesis and sensitive methods for their detection.



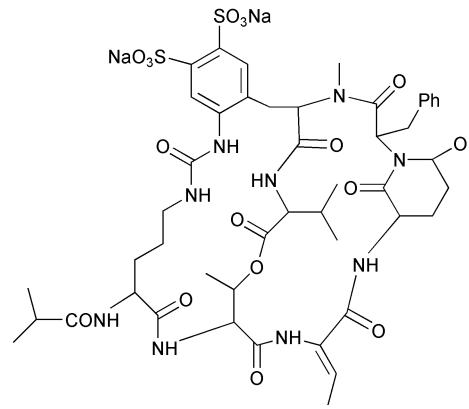
(138)



(139)



(140)



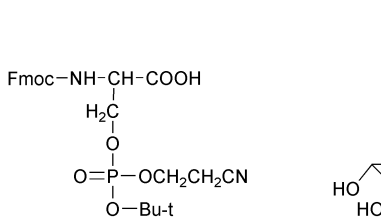
(141)

3.1 Phosphopeptides. – The two main strategies associated with phosphopeptide synthesis, the building-block approach and the global phosphorylation method, have been reviewed[149] with the active practitioner in mind. In the synthesis[150] of a triply phosphorylated pentapeptide, problems were experienced when using the building-block approach using allyloxy protection for the phosphate group. The authors' preference, to overcome the possibility that lability might be potentiated if several phosphate groups are near one another, was to carry out phosphitylation of the serine hydroxyls at the end with diallyl phosphoramidate. Subsequent oxidation with *m*-chloroperbenzoic acid and removal of a heptyl ester using a lipase gave an overall 63% yield.

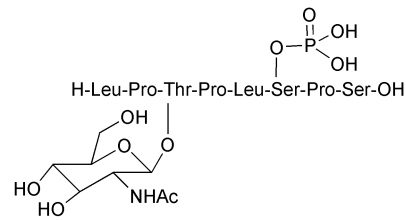
In the building-block approach it has been revealed[151] that replacing one of the t-butyl groups used for phosphate protection, with a β-cyanoethyl group as in (**142**) improves the final de-protection steps. It has been reported[152] that methyl group protection of phosphoamino acid residues is sufficiently stable to highly acidic trifluoromethanesulfonic acid to give fragments which are stable for thioester-mediated fragment condensations. Protecting groups which have been designed for removal by enzymes[153] offer mild conditions to keep the phosphate groups intact. In the synthesis of a glycophosphopeptide from human serum factor, an enzyme-labile choline ester has been used to good effect, together with the 4-(phenylacetoxy)-benzyloxycarbonyl (PhAcOZ) group which can also be removed by an enzyme. These strategies[154] were also used for the chemoenzy-

matic synthesis of a biotin-labelled analogue of (**143**) which represents a partial sequence of the c-Myc oncoprotein. Practical conditions have also been worked out[155] to enable phosphate groups attached to Thr to survive fragment condensations using the thioester approach.

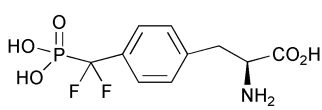
Phosphotyrosine mimetics continue to be a popular armoury for studying biological interactions. Classical organometallic reactions have been used[156] to generate the mimetic (**144**). A suitably-protected phosphotyrosine mimetic (**145**) for peptide synthesis has been produced[157] *via* a Heck reaction and incorporated into a dipeptide. X-ray crystallographic information has been used[158] to build into a short prototype sequence relating to Grb2-SH2 domain inhibitors, some mono-charged phosphinate mimetics represented by (**146**). The underlying aim was to use the additional distance provided by the methylene group in the phosphonomethyl isostere to compensate for the loss of one charge due to the additional substitution. Compounds (**146**) with R = CH_2CH_2OH, CH_2Ph and $CH_2CH(OH)Ph$ showed activities in the same range as the doubly charged compound with R = OH. Experimental details for the preparation of the 4-phosphonomethyl phenylalanine in this work has appeared in an accompanying report.[159]



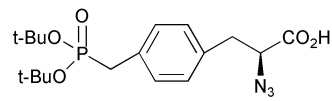
(142)



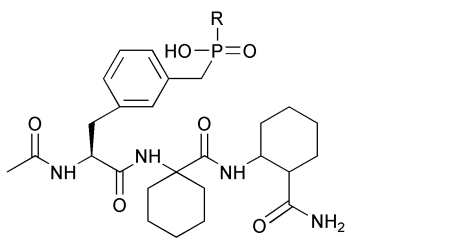
(143)



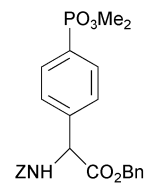
(144)



(145)



(146) R = CH_2OH, CH_2CH_2OH, CH_2Ph, CH_2CHPh, Me or OH



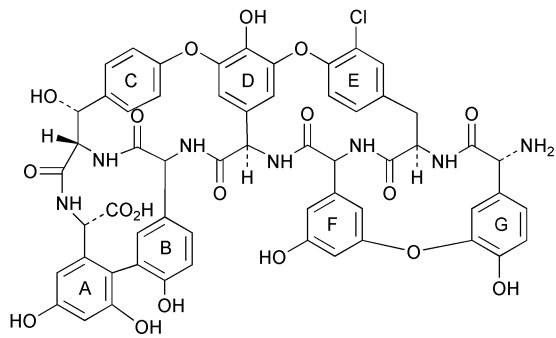
(147)

Resolution[160] of the derivative (**147**) on a gram scale, by means of a chiralcell OJ column has given enantiomerically pure samples of (+)- and (−)-forms of 4-phosphonophenylglycine (PPG). At the glutamate receptors hmGluR4a and

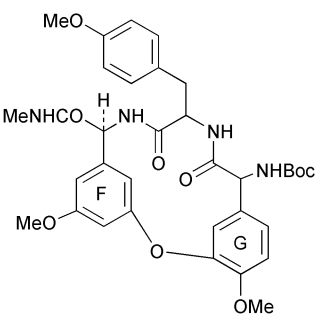
hmGluR7b only the (+)-enantiomer was active. Phosphopeptide and glycopeptide models of an antibacterial fragment (residues 173–194) of chromogranin A have been synthesised[161] but the details of the strategy were not available to the reviewer. All the models proved to be inactive against Gram positive and Gram negative bacteria.

3.2 Glycopeptide Antibiotics. – The demanding synthetic activity in this area has again been the subject of review, with compilations ranging from total synthesis[162] to more specific consideration of cycloetherification[163] and synthesis of fragments.[164]

Teicoplanin aglycone (**148**), which has the extra 14-membered F-G ring over and above the basic vancomycin nucleus, has been synthesised.[165] The common teicoplanin/vancomycin A-B-C-D ring system provided material for the first stages. Further ring condensations at the D-E rings were approached by a S_NAr diplacement using an *o*-fluoronitro aromatic ring, while macrolactamisation of the *N*-terminus amide brought about closure of the F-G 14-membered ring. Selective equilibration of the D-E ring in teicoplanin aglycones has been studied,[166] and in all cases a 1:1 mixture of P:M atropisomers was observed. An intact F-G ring system slowed down the rate of isomerisation and it was shown that the unnatural M atropisomer had significantly reduced affinity and antimicrobial activity. Exposure of a ruthenium complex of ring G to a light source promoted[167] the formation of the F-G diaryl ring, which was macrolactamised further to teicoplanin model (**149**).



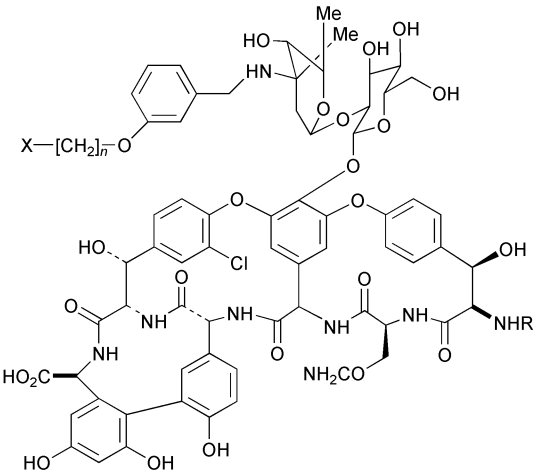
(148)



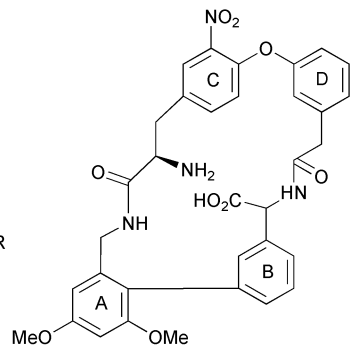
(149)

Target-accelerated combinatorial synthesis[168] of vancomycin dimers based on (**150**) has been used to identify ligands with the highest affinity for the vancomycin receptor motif, D-Ala-D-Ala. Starting with compounds such as (**150**) with $n = 2–4$; X = SCOMe, $CH=CH_2$, R = H, D-MeLeu, β-Ala, Asn *etc.*, dimerisation could be carried out by disulfide bond formation from X = SCOMe or by olefine metathesis for X = $CH=CH_2$ in both the presence and absence of D-Ala-D-Ala motifs. Three potent antibacterial agents were identified amongst the dimers. For the solid phase semi-synthesis[169] of vancomycin analogues based on modification of the oligosaccharide unit, protected vancomycin aglycone molecules have been linked to polymers *via* a selenium safety catch

linker. The main linear heptapeptide believed to be the main biosynthetic precursor of vancomycin has been synthesised[170] on solid phase. This heptapeptide D-Leu-Cyt-Asn-Hpg-Hpg-Cyt′-Dhpg where Cyt = (2*R*,3*R*)-*m*-chloro-3-hydroxytyrosine, Hpg = (*R*)-2-(*p*-hydroxyphenyl)glycine, Cyt′ = (2*S*,3*R*)-*m*-chloro-3-hydroxytyrosine and Dhpg = (*S*)-2-(3,5-dihydroxyphenyl)glycine was assembled on a chlorotrityl resin using the allyloxycarbonyl group for transient *N*-protection. When the 22-membered A-B/C-D model (**151**) and its atropostereomer was synthesised,[171] it was not possible to create the macrocyclic ring between the amino and the carboxylic acid group. The carboxyl group was able to condense with L-AlaOMe, suggesting that ring formation must be subject to some conformational strain.



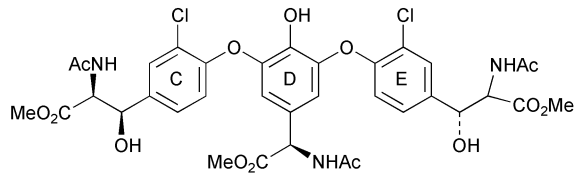
(150)



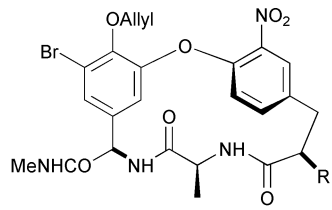
(151)

The diaryl ether network of rings C-D-E (**152**) of vancomycin have been constructed[172] using two successive oxidative coupling reactions using peroxidase. The D-E aryl ether ring was linked in the first step followed by the formation of the C-D ether link. A model (**153**) for the binding pocket of vancomycin has been synthesised[173] and its affinity for acylated alanine and lactate measured. The more H-bonding characteristics which could be included in the R group, the stronger the affinity becomes. Host–guest binding between the calix-[4]-arene vancomycin mimic (**154**) and Ac-Ala-OH and Ac-Ala-Ala-OH has been monitored.[174] The latter showed an order of magnitude higher affinity than the single amino acid derivative. Full details[175] of the synthesis and characterisation of a high affinity trivalent system derived from vancomycin and Lys-D-Ala-D-Ala have now appeared. Tris(Vancomycin carboxamide), [C_6H_3-1,3,5-($CONHC_6H_4$-4-$CH_2NHCOV)_3$, R_tV_3; V = vancomycin], binds strongly an analogous trivalent derivative of D-Ala-D-Ala, $R'_tL'_3$ (C_6H_3-1,3,5-[$CON^{\varepsilon}H(N^{\alpha}Ac)$-Lys-D-Ala-D-Ala]$_3$) in water.

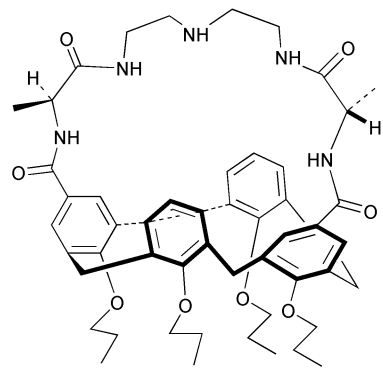
A ruthenium complex located at ring E has secured the D-E aryl ether coupling of a linear precursor to give[176] the D-E-F model framework (**155**) for ristocetin A. One approach to discovering more potent glycopeptide antibiotics



(152)



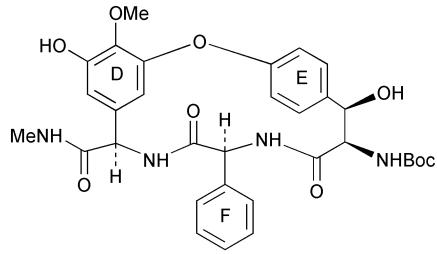
(153) R = NHBoc, $^{+}NH_3TFA^{-}$, NHCONBu-t, NHCONHCH(Me)NHBoc



(154)

is to work out their biosynthetic pathway, and to try and manipulate the producing organism to produce more potent analogues. Moves in this direction have included the expression of an α-ketoacid dioxygenase, which has been used[177] to explain the intermediacy of 4-hydroxymandelic acid in the pathway between tyrosine and 4-hydroxyphenylglycine in vancomycin. Using a *N*-methyltransferase it has also been shown[178] that *N*-methylation is a late step in the pathway to vancomycin, taking place after the oxidative crosslinking of the heptapeptide backbone.

Details of a comparison study between a solution conformation of vancomycin determined by NMR and the Protein Data Bank solid state structure have appeared.[179] A chirobiotic T column, with teicoplanin as the chiral stationary phase, separates[180] in one run the four stereoisomers of cyclic β-substituted α-amino acids, such as 1-amino-2-methyl cyclohexane. In a separate study[181] the role of the different parts of teicoplanin in chiral separation has been considered and the conclusion drawn that it is the aglycone part which contributes to the enantioseparation of amino acids. Non-amino acid species seem to be separated



(155)

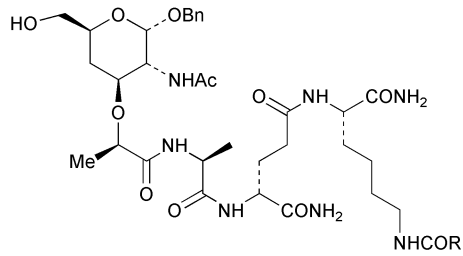
better with the carbohydrate moiety present on the teicoplanin. Insertion[182] of lipophilic substituents at the C-6 glucose position of the sugar residue in vancomycin has given rise to analogues with more potency than the parent antibiotic.

Dimerisation constants for vancomycin, ristocetin and eremomycin and its analogues have been ascertained[183] using electrospray mass spectrometry. The results correlated well with previously reported values from NMR measurements. Optimum antibiotic activity in bleomycin is realised when iron is bound to its structure. A detailed spectroscopic and theoretical study[184] of the ferric form of the drug and its activated analogue (the hydroperoxide form) has now been completed. The results were extended to include a possible explanation to the drug's interaction with DNA, either *via* a homolytic cleavage of a hydroperoxide to give Fe(IV) bleomycin complex and an OH radical or a Fe(IV) complex and a DNA radical.

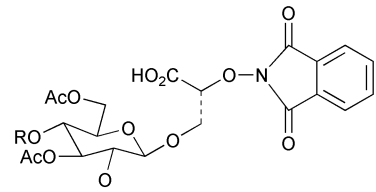
3.3 Glycopeptides. – There are again sufficient specialist papers published under each section to maintain the O-, N-, and C-linked glycopeptide sub-divisions. However, certain aspects have cross-divisional appeal and are discussed here. One German group of experts in this field have written three separate reviews[185–187] emphasising synthetic schemes both in the solution and solid phases, and covering the publications up to 1999. The preparation and conformational properties of glycopeptides have been reviewed[188] and a book emphasising practical aspects has a chapter on glycopeptide synthesis.[189] Recent progress in the solid phase synthesis of glycopeptides has been reviewed[190] as well as the assembling of combinatorial libraries[191] for oligosaccharides and glycopeptides. Selective chemical modification of glycopeptide antibiotics has also been the subject of a short review.[192]

A combinatorial library based on variations in the R group of the muramyl dipeptide derivative (**156**) has been set up[193] using a multipin approach and facile synthesis[194] of *N*- and *O*-glycosylated α-aminooxy acids such as (**157**) will enable studies on novel polymeric structures to be carried out. The synthesis[195] of orthogonally-protected glucose units such as (**158**) and (**159**) has enabled multivalent glycopeptide mimetics to be prepared and have the potential to make glycopeptide dendrons. A study[196] involving the synthesis of templates such as (**160**) has successfully demonstrated that glycosylation reactions [*i.e.* disaccharide formation in (**160**)] may be achieved between glycosyl donors and acceptors

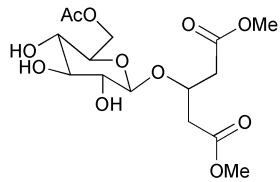
that are bound to peptide templates. Modest regio-stereoselectivities can be achieved in this way.



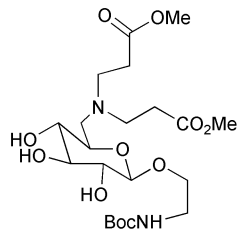
(156)



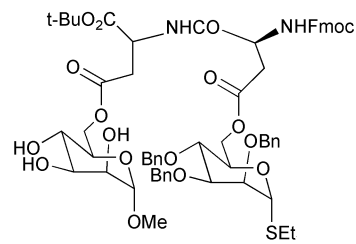
(157)



(158)



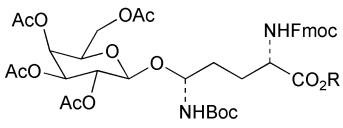
(159)



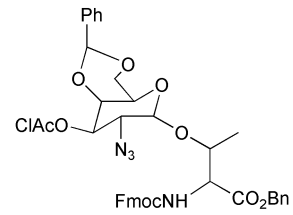
(160)

3.3.1 O-Glycopeptides. There is a continuing demand for protected glycosylated building blocks for solid-phase and combinatorial work. An improved synthesis[197] of the protected hydroxylysine derivative (**161**) is therefore welcome. Key to a successful 80% yield was the use of Fmoc-Hyl(*Z*)-OAll as starting material. Addition of cyclodextrins has enhanced[198] the solubility of Fmoc-Thr(GalNAcα1)-OBu^t up to 100-fold. This has enabled glycan chain extension using enzymes to be carried out in a one-pot reaction giving, as an example, Fmoc-Thr[Neu5Ac(α2-3)Gal(β1-3)GalNAcα1]-OBu^t in 50% yield. A phenylseleno glycosyl donor has been coupled[199] to Fmoc-Thr-OBn to give the α-linked building block (**162**). Different sialic acid-containing building blocks *e.g* (**163**) and (**164**) have been prepared[200] by using glycosyl xanthates as donors for the threonyl derivatives under catalysis by phenyl sulfanyl triflate.

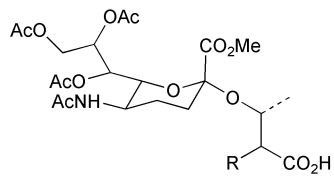
Building blocks, Fmoc-Tyr-OH and Fmoc-(ahα-GalNAc)$_3$Glu-OH were used[201] to synthesise the ligand (**165**), known to have a sub-nanomolar affinity for asialglycoprotein receptor. A protected glycine derived phosphonate has been reacted[202] with a n-pentenyl glycoside to stereoselectively form the glyco-amino acid derivative (**166**). The N-terminal fragment of human glycophorinAM bearing the consecutive sialyl-T antigen [α-D-Neu5Ac(2-3)-β-D-Gal(1-3)α-D-GalNAc] has been synthesised[203] *via* the protected intermediate (**167**) which is based on stepwise assembly of glycosylated Fmoc-amino acids bearing the appropriate R group. In an attempt to identify[204] optimal Lewis^y-based anti-cancer vaccine



(161)



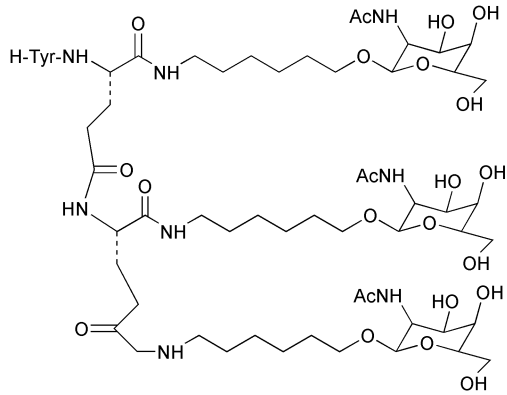
(162)



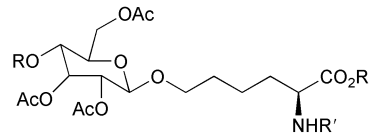
(163) R = Fmoc
(164) R = N_3

leads, a series of glycopeptide mimics, (**168–172**), of cell-surface Lewis^y mucin glycoproteins have been synthesised on solid phase. A β-turn mimetic (**173**) of sialyl Lewis^x, which is a natural carbohydrate ligand of the cell-adhesion molecules, the selectins has been synthesised[205] by solid phase techniques. Tested against E-selectin, compound (**173**) showed only weak activity, but it showed more potent inhibitory activity towards P- and L-selectin. The procedure of linking an *O*-silylated Fmoc-Thr(GalNAc)-OAll residue *via* its side chain onto a resin (procedure published in 1999) has been used[206] as the first stage in the solid phase synthesis of the N-terminal glycodecapeptide of interleukin 2. The silyl ether linker could be cleaved by fluoride or acid. The building block Fmoc-Thr(or Ser)[α-Neu5Ac-(2-3)-β-Gal(1-3)-α-GalN_3(1-)]OPfp has been used[207] in the synthesis of the sialyl T decapeptides (**174**). In contrast to analogues containing T-antigens, these sialyl-T-glycopeptides were non-immunogenic, indicating that sialylation might be a method of circumventing recognition by the immune system.

The disulfide-bridged glyco forms (**175**) of catfish somatostatin (somatostatin-22) have been synthesised[208] starting from Fmoc-Cys(Trt)-PAC-PEG-PS support. The glyco links were carried through the assembly of residues as acetylated glycosidic building blocks linked to threonine, while the disulfide ring was synthesised in the solution phase. There was a 2-fold increase in binding to the receptor when the R group in (**175**) was a disaccharide. Established methods of solid phase synthesis, incorporating the glyco links either as Fmoc-Ser(αGalNAc)-OH or Fmoc-Thr(αGalNAc)-OH have been used[209] for short synthetic glycopeptide sequences to induce antibody responses to carcinoma-associated Tn antigens. These sequences were recognised by Tn-specific monoclonal antibodies, and it seemed that the titres were not altered by changes in the peptide backbone. A combination of a newly-designed allyl linker attached to a Sieber amide resin has enabled[210] Ser(glycosyl)-Gly repeating sequences up to hexadecapeptide in size to be synthesised. In the synthesis[211] of a sialyl T-antigen linked tetrapeptide (**176**), the peptide portion with GalNAc attached to Ser was assembled on-resin. The side chain saccharide was then subjected to a recom-

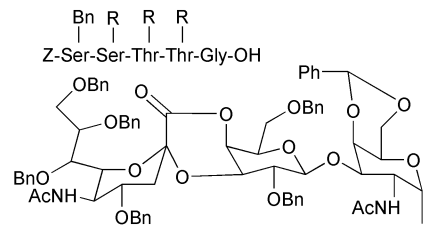


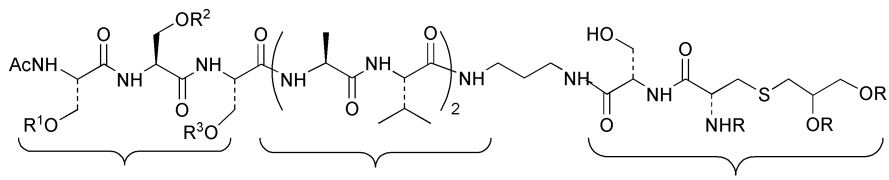
(165)



(166)

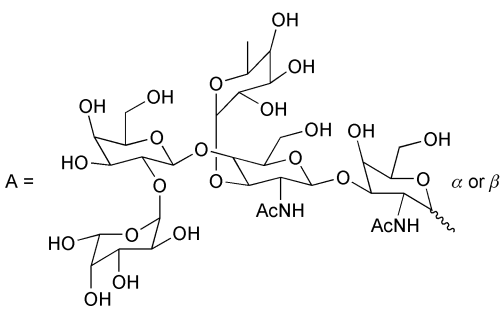
(167) R =



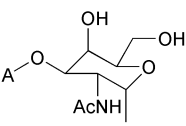


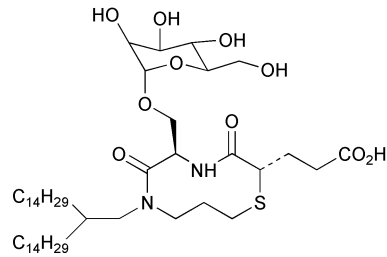
(168) $R^1 = R^2 = R^3 = \alpha$ A
(169) $R^1 = R^2 = R^3 = \beta$ A
(170) $R^1 = R^2 = R^3 = \alpha$ A
(171) $R^1 = R^2 = R^3$ = B
(172) $R^1 = R^2$ = H R^3 = B

A =

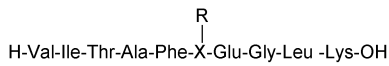


B = β—A

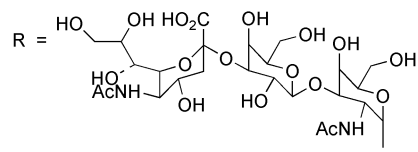




(173)



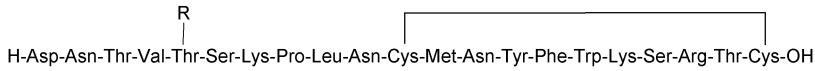
(174) X = Thr or Ser



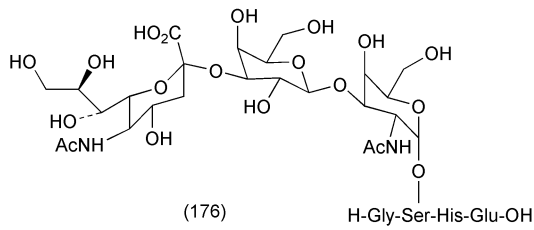
binant β-galactosidase to add the second residue, followed by sialyltransferase catalysed addition of the final residue to form (**176**). Exploratory studies[212] using chemo-enzymatic strategies for the synthesis of the N-terminal sequence (**177**) of P-selectin glycoprotein ligand-1 (PSGL-1) have led to a semi-preparative scale protocol[213] being developed. The first stages involves assembly of the peptide with a disacharide unit attached to Thr, followed by addition of the sulfate using the SO_3-pyridine complex. A glycosyltransferase-catalysed completion of the sialyl Lewis^x side chain was then carried out without detriment to the sulfate group.

The base-catalysed removal of the glycosidic acetyl groups from synthesised glycopeptides, Ac-Ala-Ser[β-Gal$(OAc)_4$]-Phe-NH_2, Ac-Ala-MeSer[β-Gal$(OAc)_4$]-Phe-NH_2 and Ac-Ala-Ser[β-Gal$(OAc)_4$]-MePhe-NH_2, has been studied.[214] The glycopeptide carrying the MeSer residue was more susceptible to β-elimination than the others, confirming the observation that aza-enolates formed from the amide bond to a glycosylated Ser provide protection against β-elimination. In order to study[215] the mechanism of action proposed for the glycolipo-depsipeptide ramoplanin, the model compound (**178**) has been synthesised. Reducing the size of the lipid side chain has created a more water-soluble analogue which could be used in a modified membrane assay.

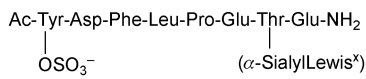
The δ-selective glycosylated enkephalin amide H-Tyr-D-Thr-Gly-Phe-Leu-Ser(β-D-Glc)-NH_2 has been synthesised[216] using Fmoc-Ser[β-D-Glc$(OAc)_4$] as a key building block. It compared favourably as an agonist with the un-glycosylated form, but showed improved intracerebroventricular and intraventricular properties. When the NMR data for the glycosylated enkephalins (**179**) were compared[217] with their non-glycosylated form, it was found that residues within the disulfide ring were not affected by glycosylation. However, the exocyc-



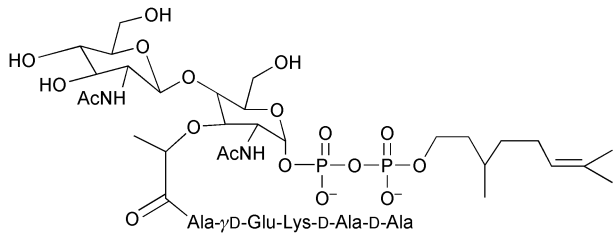
(175) R = α D-GalNAc or R = β-D-Gal(1-3)-α-D-GalNAc



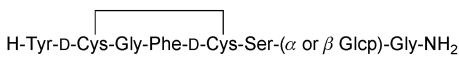
(176)



(177)



(178)

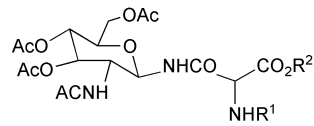


(179)

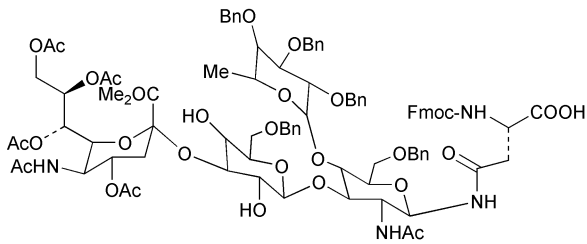
lic Ser-Gly residues were affected, especially so, when the Ser-carbohydrate link was β. Details of a strategy[218] to synthesise glycosylated hydroxylysine derivatives suitable for the Fmoc-building block approach have been revealed. They involve introduction of a β-D-galactopyranose group to a copper-complexed Hyl(Boc or Aloc), followed by stepwise conversion to Fmoc-Hyl[Boc or Aloc, *O*-β-D-Gal(OAc)$_4$]. The Boc derivative was used in the synthesis of the α1(IV) 1266-1277 sequence from type (IV) collagen.

3.3.2 N-Glycopeptides. Synthesis of suitable derivatives as protected building blocks for solid phase assembly has also been undertaken in this category. Thus the formation of an *N*-glycosidic link between GlcNAc and Asn has been effected[219] using the γ-acid fluoride of a protected aspartic acid and either a glycoside azide or silyl carbamate to give (**180**). The sialyl Lewis-asparagine building block (**181**) has been synthesised[220] and used[221] in the synthesis of the

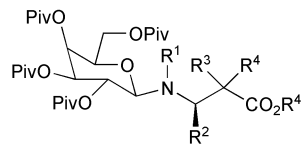
partial sequence of the binding region of PSGL-1 in which the *O*-glycosyl Thr is replaced by the deprotected form of (**181**). Post-synthetic purification using HPLC has enhanced the diastereomeric purity of a sample of the glycosylated β-amino acid ester (**182**). Building blocks such as (**182**) based on β-amino acids have been developed[222] *via* a Mannich type reaction between *O*-pivaloylated *N*-galactosyl imines and silyl ketene acetals.



(180)



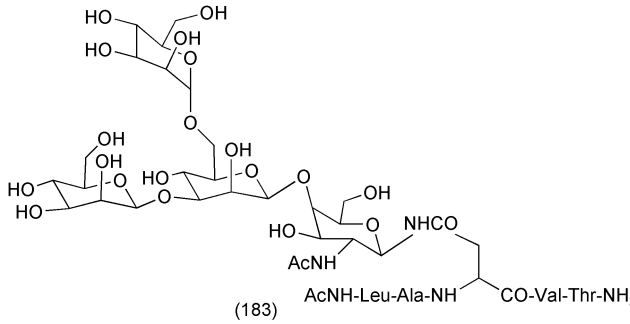
(181)



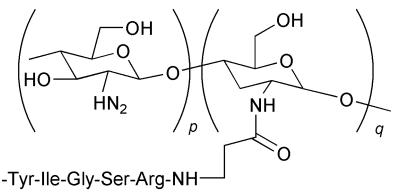
(182)

Key to the synthesis[223] of the mannose-rich glycopeptide (**183**) was the condensation between an unprotected glycosyl amine and a protected aspartyl-containing peptide. The other saccharide units were added on stereoselectively. A conjugate between a laminin-related peptide and chitosan has involved construction of an amide link between saccharide and peptide. 6-*O*-Trityl chitosan has been coupled[224] *via* a glucosamine link to afford the conjugate (**184**). This had a higher inhibitory activity against experimental lung metastasis of B16BL6 melanoma cells in mice than the parent peptide. A heptasaccharide attached to asparagine, [α-D-Man(1-6)-[α-D-Man(1-3)]-[2-AcNH-2-deoxy-β-D-Glc(1-2)]-(β-D-Man)-(1-4)-(α-1-Fuc)-(1-6)]-2-AcNH-2-deoxy-β-D-Glc]-Asn, has been synthesised[225] using *p*-methoxybenzyl-assisted intramolecular aglycone delivery as the key transformation.

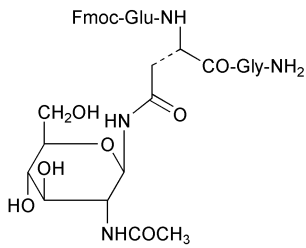
The glycopeptide (**185**) was assembled[226] without protection of the carbohydrate hydroxyls, on a continuous surface of cellulose (SPOT synthesis). Fmoc-Gly-OPfp was first coupled to the amine groups on the solid surface and after deprotection, glycosylated-Asn and -Glu residues were added in turn. Cleavage from the continuous surface was by UV irradiation giving (**185**) in 90% yield. Attachment of 2,3,4-tri-OAc-1-azido-1-deoxy-β-D-glucopyranuronic acid to the



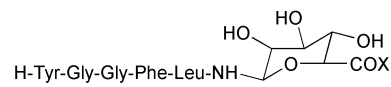
(183)



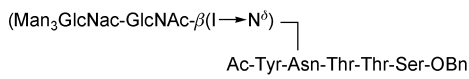
(184)



(185)



(186) X = Polymer, NH_2 or OH

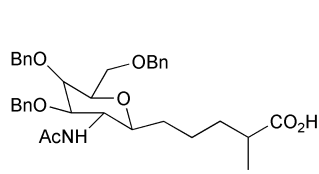


(187)

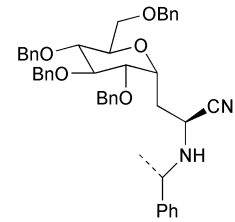
resin *via* X in formula (**186**) was the initial step used[227] for the synthesis of C-terminal glycopeptides based on the enkephalin sequence. Condensation[228] of the β-COOH group of Asp in the protected assembled pentapeptide, with a heptasaccharide glycosylamine, *via* a HOBt ester in the solution phase, has yielded (**187**). NMR-ROESY experiments and molecular dynamics simulations on (**187**) indicate that the carbohydrate moiety seems to give less conformational mobility to the peptide backbone.

3.3.3 C-Linked and Other Linked Glycopeptides. This area of glycopeptidomimetics has been the subject of a specialist review[229] on compounds with amino acids linked to the anomeric carbon of the sugar *via* C–C bonds. Mimicking[230] the *N*-glyco link to Asn as an ethylene group is now possible due to the synthesis of (**188**) in either α or β form. The bond was constructed by reaction of

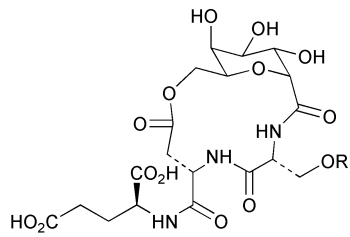
lithium *C*-glycosyl acetylides with Boc-D-serinal acetonide. In an attempt[231] to synthesise amino acid functions closer to the sugar moiety, it was found that a Strecker reaction between an aldehydo-sugar, acetone cyanohydrin and a benzylamine gave the cyanide (**189**), which could be readily hydrolysed to the corresponding carboxylic acid. A C-linked isostere of β-D-galactosylated hydroxynorvaline has required[232] eight steps for its synthesis from per-*O*-benzylated galactopyranolactone. Key steps involved adding homoallylic Grignard reagent to the lactone, reduction of the hemiacetal formed using triethylsilane, followed by a Wittig reaction with Garner's aldehyde. This C-linked building block became residue 264 in the fragment CII(256-270) from type II collagen. However, this one change of a methylene group for an oxygen atom in the carbohydrate moiety reduced the T-cell response by 10–20-fold.



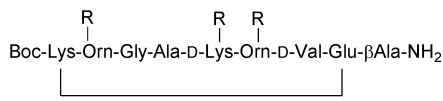
(188)



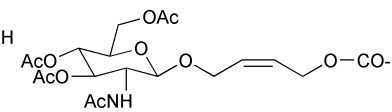
(189)



(190)



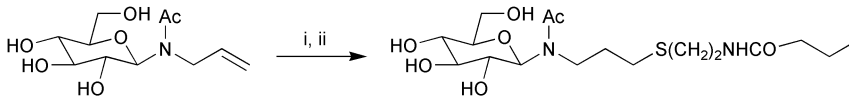
(191) R = H
(192) R =



Two novel *C*-glycosides of aureolic acid linked to oliose and olivose have been isolated[233] from *Streptomyces* sp. Macrocycle (**190**) has been designed[234] and synthesised as a mimic of the tetrasaccharide SLe^x inhibitor of P-selectin. A 1000-fold increase in potency (IC_{50} = 1 μm) over SLe^x was found for (**190**). Libraries of glyco-clusters have been based[235] on the acylation of cyclic compounds (**191**), by their corresponding R ligands using *p*-nitrophenylcarbonates to make compounds such as (**192**). Minimal modification of the saccharide was

required for the preparation[236] of a neoglycopeptide building block (**193**), using the stepwise processes summarised in Scheme 3. Lower molecular weight neoglycopeptides, typically based on Cys-Trp-Lys_{18} coupled to lactose, galactose or mannose *via* iodoacetic acid, have been characterised[237] using NMR data. The glycopeptides formed DNA condensates of approximately 110 nm mean diameter with zeta potential of +31 mV.

S-Glycoside amino acid derivatives are known to be more resistant to chemical and enzymatic degradation and hence the interest in them as building blocks

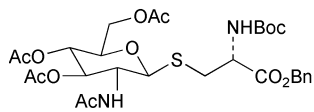


(193)

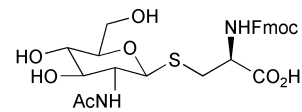
Reagents: i, *hν*, $NH_2CH_2CH_2SH$; ii, transglutamine-catalysed

Scheme 3

for glycopeptidomimetics. Building block (**194**) has been synthesised[238] either from a sugar thiol and a serine derivative using a Mitsunobu reaction or from a 1-pseudo thiourea of GlcNAc and iodo-Boc-Ala-Bn. *S*-Glycoamino acid derivative (**195**) was obtained[239] from an iodine activated Fmoc derivative and a sugar 1-thiolate.



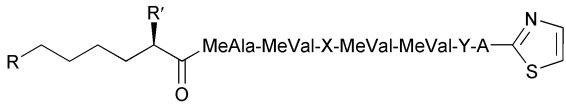
(194)



(195)

3.4 Lipopeptides. – Apramides A-G have been isolated[240] from the cyanobacterium *Lyngbya majuscula* and have been characterised as (**196–202**) respectively. NMR evidence showed distinguishable conformers in solution, attributable to the thiazole C-terminal unit. The characterisation[241] of four new friulimicins A, B, C, and D from the cultures of *Actinoplanes friuliensis* HAG 010964 has made it possible to realign the structures with other amphomycin type lipopeptides present in the culture fluid. The full structural list appears in (**203–210**). Assuming that the published antibiotics, amphomycin, tsushimycin, glumamycin and aspartocin all have the same peptide framework, then (**204**), (**207**) and (**209**) are identical to tsushimycin, amphomycin and aspartocin respectively.

Developments in the use of enzyme protecting group techniques in the synthesis of lipidated peptides have been reviewed.[242] The Kaiser oxime resin has been used[243] in the solid phase synthesis of a library of farnesylated peptides represented by (**211**). This molecule proved to be the most potent inhibitor of Ras CaaX endoprotease. In a re-synthesis[244] of antillatoxin (**212**) to form the (4*R*,5*R*) configurated molecule as shown, proof was obtained that the natural compound was identical with the synthetic sample. An N-terminal, myristoylated and twice *S*-palmitoylated 29-mer peptide from the N-terminus of endothelial NO-synthase has been synthesised.[245] For the *S*-palmitoylated building blocks, the



(196) R = —≡, R′ = Me, X = Pro, Y = DiMeTyr, A = —N—CH_2—

(197) R = —≡, R′ = H, X = Pro, Y = DiMeTyr, A = —N—CH_2—

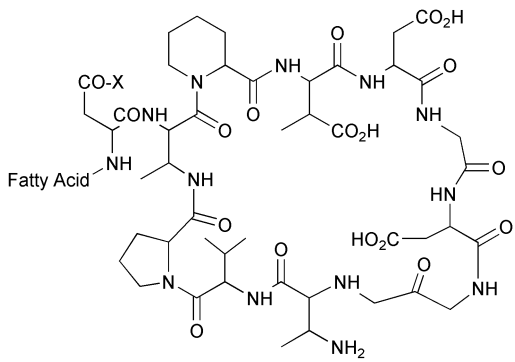
(198) R = —≡, R′ = Me, X = Pro, Y = DiMeTyr, A = —N—CH_2—

(199) R = —≡, R′ = Me, X = Pro, Y = DiMeTyr, A = —N

(200) R = —≡, R′ = H, X = Pro, Y = DiMeTyr, A = —N

(201) R = —≡, R′ = Me, X = Pro, Y = DiMeTyr, A = —N

(202) R = —≡, R′ = Me, X = MeVal, Y = no residue, A = —N

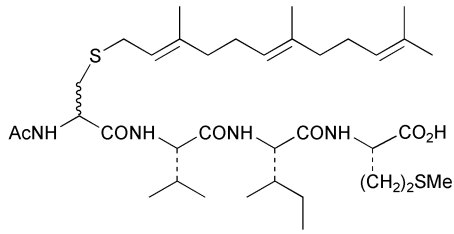


	Name	X	Fatty Acid
(203)	A 1437A	OH	Δ3-isotridecenoic acid
(204)	A 1437B	OH	Δ3-isotridecenoic acid
(205)	Friulimicin A	NH_2	Δ3-isotridecenoic acid
(206)	Friulimicin B	NH_2	Δ3-isotridecenoic acid
(207)	A 1437E	OH	Δ3-anteisotridecenoic acid
(208)	Friulimicin C	NH_2	Δ3-anteisotridecenoic acid
(209)	A 1437G	OH	Δ3-anteisopentadecenoic acid
(210)	Friulimicin D	NH_2	Δ3-anteisopentadecenoic acid

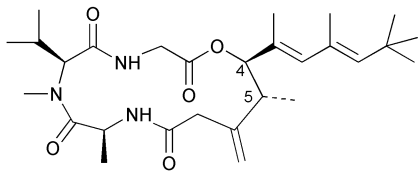
enzyme-labile PhAcOZ group and allyl esters were used. *N*-Myristoylated fragments were synthesised by Fmoc-solid phase techniques on a chlorotrityl resin. Fragments were coupled together with EDC/HOAt in DMSO to aid solubility. The palmitoyl/glyoxyl unit (**213**) reacts[246] under salt-free conditions with N-terminal α-hydrazino acetyl peptides to give a hydrazone ligation product.

Three highly lipophilic mesityl-substituted amino acid derivatives such as α-mesityl glycine (**214**) have been synthesised[247] by enantioselective routes, since

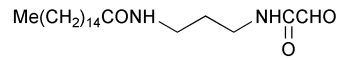
enzymic resolution cannot cope with this unusual substitution. For (**214**) and the other β-mesitylalanines synthesised, NMR data and semi-empirical calculations show that the mesityl group imposes strong conformational restrictions on the molecules. FK463, a new echinocandin-like lipopeptide, in phase II/III clinical trials, has been tested[248] against various fungal pathogens. The results showed promise as a treatment for fungal infections.



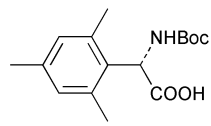
(211)



(212)



(213)



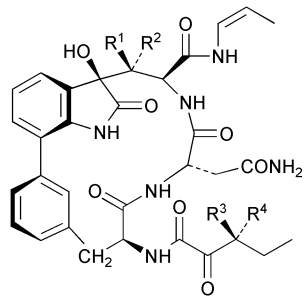
(214)

4 Miscellaneous Structures

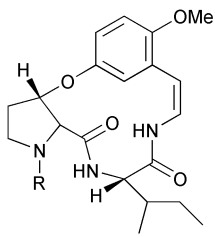
Molecules from nature itself, some man-made 'designer' molecules and macrocyclic forms which serve as pro-drugs have been included in this section, mainly because they fall uneasily into the sub-divisions above. This does not demote their importance to the scientific literature.

Four new proteasome inhibitors, TMC-95 (A–D) have been isolated[249] from the fermentation broth of *Apiospora montagnei* Sacc TC 1093 and characterised as (**215–218**). Physico-chemical data revealed a planar structure, with the congeners in essence being diastereoisomers of one another. The roots of *Paliurus ramossisimus* have produced[250] seven new cyclopeptide alkaloid structures (**219–225**) designated paliurines A–F respectively. A first total synthesis[251] of mauritine A (**226**) has been accomplished by combining the aryl-alkyl ether bond formation and macrocyclisation into a single operation using a S_NAr reaction.

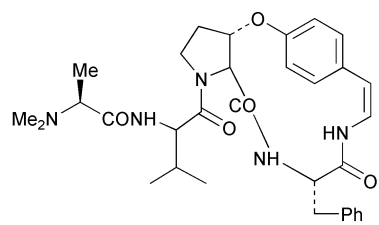
Designer cyclopeptides which function as synthetic receptors for biological substrates have become fashionable. Macrobicyclic structures[252] such as (**227**) have been analysed by X-ray crystallography and has been found to be an



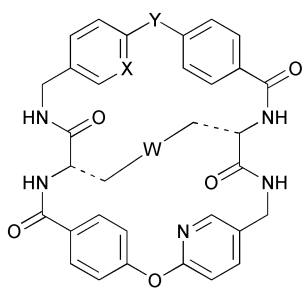
	R^1	R^2	R^3	R^4
(215)	H	OH	Me	H
(216)	H	OH	H	Me
(217)	OH	H	Me	H
(218)	OH	H	H	Me



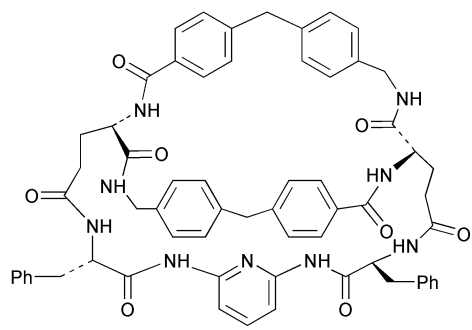
(219) R = DiMeIle-Phe
(220) R = MeIle-Phe
(221) R = DiMeIle
(222) R = DiMePhe-Ile
(223) R = MePhe-Ile
(224) R = DiMePhe
(225) R = DiMeleu-Ile



(226)

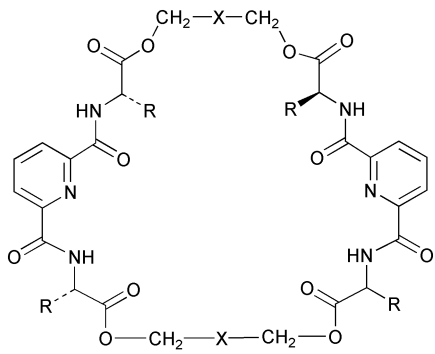


(227) X = N, Y = O, W = $NHCSNH(CH_2)_3$ or
X = CH, Y = CH_2, W = $NHCSNH(CH_2)_3$

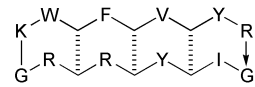


(228)

effective receptor for *N*-acetyl asparagine, while the binding properties[253] of (**228**) have been determined by NMR techniques. Reasonable structural and stereo selectivity have been detected for dipeptides such as *Z*-β-Ala-D-Ala and the nature of the association deduced from NMR and modelling studies. Pyridine-based, polyamido-polyester macrocycles such as (**229**) and smaller cyclic ring analogues have been synthesised[254] and their conformation determined by X-ray crystallography. Molecule (**229**) showed rigidity and strain in its structure and exhibits significant chiral recognition for the enantiomers of D- and L-amino acid methyl ester hydrochlorides. With recent examples (*e.g.* tachyplesins) of highly rigid cyclic β-stranded antimicrobial peptides being found in nature, design of analogues is underway. Analogues of (**230**) have been synthesised[255] in a parallel series, by replacement of the bulky hydrophobic residues in turn with Gly residues. The final macrocyclisation was *via* the Tam thioester 'thia zip' cyclisation. The Gly_6 analogue of (**230**) retained broad-spectrum activity against all 10 test microbes in both low and high salt concentrations. The adamantane-constrained cyclo $(Adm\text{-}Cys)_3$ has undergone X-ray analysis[256] which revealed a double-helical (figure of eight) fold in the macrocycle, stabilised by two intramolecular H-bonds.



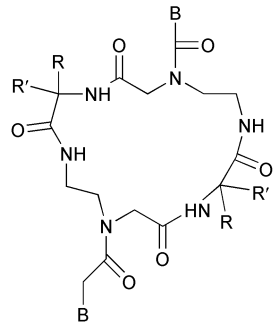
(229)



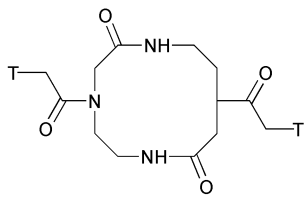
(230) ····· disulfide bonds

Cyclic peptide hybrids, such as (**231**) with nucleobases alternating with amino acid side chains on the cyclopeptide backbone have been assembled[257] on solid phase or by cyclisation of the linear precursors in solution. PyAOP/HOAt/DIEA were the reagents of choice for the cyclisation. Various conditions have been explored[258] for the cyclisation of bis-aminoethyl glycinamide frameworks, which can then be acylated with a nucleobase acetyl function to give cyclic peptide nucleic acids such as (**232**). Cyclisation from suitably protected pentafluorophenyl ester precursors proved to be an efficient process.

The structural core (**233**) of antimitotics such as phomposin and ustiloxin has been synthesised[259] using an S_NAr cycloetherification reaction starting from an aryl *ortho*-nitro fluoro precursor. It has been shown[260] that in solid phase synthesis of the 1-thio-4-nitro substituted structure (**234**), cyclisation *via* S_NAr reactions is more efficient than its 2-thio-5-nitro counterpart. Compound (**234**) had been designed to be a β-turn mimic whereas its isomer would not show such

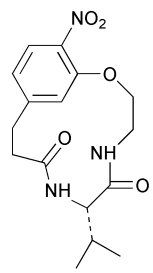


(231) B = nuleobases, A, T, C or G
R, R′ = H or amino acid side chains

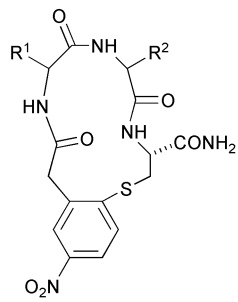


(232)

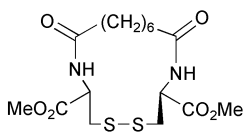
conformational preference. This was borne out in the physical data accumulated, with molecular dynamics simulations showing supporting evidence for the easier formation of (**234**) over its isomer. The cyclobisamide (**235**) and its bis-urea analogue can be prepared[261] in a one-step condensation between octan-1,8-dicarbonyl chloride or diisocyanate with appropriate Cys-Cys derivatives. X-ray studies show that the molecules have the inherent property of self-assembly into hollow tubular structures. Construction of the ring in compound (**236**) by forming the double-bond using ruthenium complexes gives an array[262] of cyclic sulfamide peptidomimetics, while the principle of folding a linear biologically active peptide domain into an esterase sensitive cyclic pro-drug model has been reviewed.[263] As an example[264] of the latter approach, the hexapeptide, H-Trp-Ala-Gly-Gly-Asp-Ala-OH, from δ-sleep-inducing peptide has been cyclised, *via* an acyloxy alkoxy bridge as in (**237**), which then re-releases the hexapeptide in the presence of an esterase.



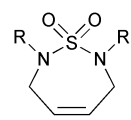
(233)



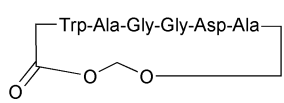
(234) $R^1 = CH_2CONH_2$, $R^2 = (CH_2)_4NH_2$



(235)



(236)



(237)

5 References

1. C. A. Selects on *Amino Acids*, *Peptides and Proteins*, published by the American Chemical Society and Chemical Abstracts Service, Columbus, Ohio.
2. *The ISI Web of Science Service for UK Education* on http://wos.mimas.ac.uk.
3. *Peptides for the New Millennium* (Proceedings of the 16th American Peptide Symposium), G. B. Fields and G. Barany eds. Kluwer, Dordrecht, Netherlands, 2000, 829 pp.
4. D. J. Faulkner, *Nat. Prod. Rep.*, 2000, **17**, 1; 7.
5. *Fmoc Solid Phase Peptide Synthesis*: *A Practical Approach*, W. C. Chan and P. D. White eds., Oxford University Press, 2000, 346 pp.
6. A. F. Spatola and P. Romanovskis in *Amide Linkage*, A. Greenberg, C. M. Breneman and J. F. Liebman, eds., J. Wiley and Sons, NY, 2000, pp. 519–564.
7. J. P. Tam, Q. Yu and Z. Miao, *Biopolymers*, 2000, **51**, 311.
8. H. Heimgartner, F. S. Arnhold, S. P. Fritschi, K. N. Koch, J. E. F. Magirius and A. Linden, *J. Het. Chem.*, 1999, **36**,1539.
9. T. Johnson, M. Liley, T. J. Cheeseright and F. Begum, *J. Chem. Soc. Perkin Trans. 1*, 2000, 2811.
10. D. L. Rabenstein, T. Shi and S. Spain, *J. Am. Chem. Soc.*, 2000, **122**, 2401.
11. G.-J. Chen, H.-W. Tan and R.-Z. Liu, *Huaxue Xuebao*, 2001, **59**, 62.
12. A. G. Kozlovskii, N. G. Vinokurova and V. M. Adanin, *Prikl. Biokhim. Mikrobiol.*, 2000, **36**, 317.
13. A. G. Kozlovskii, N. G. Vinokurova, V. M. Adanin, G. Burkhardt, H.-M. Dahse and U. Gräfe, *J. Nat. Prod.*, 2000, **63**, 698.
14. X. Fu, S.-Y. Su and L.-M. Zeng, *Chin. J. Chem.*, 2000, **18**, 882.
15. Z. Ding, J. Zhou and N. Tan, *Zhongcaoyao*, 2000, **31**, 803.
16. S. Jeedigunta, J. M. Krenisky and R. G. Kerr, *Tetrahedron*, 2000, **56**, 3303.
17. H. Kanzaki, D. Imura, T. Nitoda and K. Kawazu, *J. Antibiotics*, 2000, **53**, 58; H. Kanzaki, D. Imura, T. Nitoda and K. Kawazu, *J. Biosci. Bioeng.*, 2000, **90**, 86.
18. F. von Nussbaun and S. J. Danishefsky, *Angew. Chem. Int. Ed.*, 2000, **39**, 2175.
19. P. R. Sebahar and R. M. Williams, *J. Am. Chem. Soc.*, 2000, **122**, 5666.
20. H. Wang and A. Ganesan, *J. Org. Chem.*, 2000, **65**, 4685.
21. L. E. Overman and M. D. Rosen, *Angew. Chem. Int. Ed.*, 2000, **39**, 4596.
22. H. Wang, T. Usui, H. Osada and A. Ganesan, *J. Med. Chem.*, 2000, **43**, 1577.
23. J. F. Sanz-Cervera, E. M. Stocking, T. Usui, H. Osada and R. M. Williams, *Bioorg. Med. Chem.*, 2000, **8**, 2407.
24. Y. Hayashi, S. Orikasa, K. Tanaka, K. Kanoh and Y. Kiso, *J. Org. Chem.*, 2000, **65**, 8402.
25. J. S. Davies, M. Stelmach-Diddams, R. Fromentin, A. Howells and R. Cotton, *J. Chem. Soc. Perkin Trans. 1*, 2000, 239.
26. A. Bianco, C. P. Carsten, P. Roepstorff and J.-P. Briand, *J. Org. Chem.*, 2000, **65**, 2179.
27. A. Bianco, J. Furrer, D. Limal, G. Guichard, K. Elbayed, J. Raya, M. Piotto and J.-P. Briand, *J. Comb. Chem.*, 2000, **2**, 681.
28. N. V. S. Ramakrishna, T. S. More, Y. Khandelwal, R. G. Naik, B. Lal, R. D. Gupte and R. V. S. V. Vadlamudi, *Ind. J. Chem. Sect. B*, 1999, **38B**, 1331.
29. L. M. Van Langen, F. Van Rantwijk, V. K. Svedas and R. A. Sheldon, *Tetrahedron*: *Asymmetry*, 2000, **11**, 1077.
30. P. Leeming, F. R. Fronczek, D. J. Ager and S. A. Laneman, *Top. Catal.*, 2000, **13**, 175.

31. S. Jin, P. Wessig and J. Liebscher, *Eur. J. Org. Chem.*, 2000, 1993.
32. T.-J. M. Luo and G. T. R. Palmore, *J. Phys. Org. Chem.*, 2000, **13**, 870.
33. K. Hanabusa, M. Matsumoto, M. Kimura, A. Kakehi and H. Shirai, *J. Colloid Interface Sci.*, 2000, **224**, 231.
34. F. L. Bettens, R. P. A. Bettens, R. D. Brown and P. D. Godfrey, *J. Am. Chem. Soc.*, 2000, **122**, 5856.
35. Y.-D. Cheng and S.-Y. Lin, *J. Agric. Food Chem.*, 2000, **48**, 631.
36. O. El Mahdi, J.-P. Lavergne, J. Martinez, P. Viallefont, E. M. Essassi and C. Riche, *Eur. J. Org. Chem.*, 2000, 251.
37. J. F. Reichwein and R. M. J. Liskamp, *Eur. J. Org. Chem.*, 2000, 2335.
38. A. Bigot, M. Bois-Choussy and J. Zhu, *Tetrahedron Lett.*, 2000, **41**, 4573.
39. M. Pérez-Gonzalez and R. F. W. Jackson, *Chem. Commun.* (*Cambridge*), 2000, 2423.
40. Y. Masuoka, K. Shin-Ya, K. Furihata, H. Matsumoto, Y. Takebayashi, K. Nagai, K.-I. Susuki, Y. Hayakawa and H. Seto, *J. Antibiotics*, 2000, **53**, 793.
41. S. L. Colletti, C. Li, M. H. Fisher, M. J. Wyvratt and P. T. Meinke, *Tetrahedron Lett.*, 2000, **41**, 7825; P. T. Meinke, S. L. Colletti, M. B. Ayer, S. J. Dorkin-Rattray, R. W. Myers, D. M. Schmatz, M. J. Wyvratt and M. H. Fisher, *Tetrahedron Lett.*, 2000, **41**, 7831; 7837.
42. B. Anichini, R. Ricci, G. Fabbri, G. Balacco, S. Mauro, A. Triolo, M. Altamura, C. A. Maggi and L. Quartara, *J. Pept. Sci.* 2000, **6**, 612.
43. M. El Haddadi, F. Cavalier, E. Vives, A. Azmani, J. Verducci and J. Martinez, *J. Pept. Sci.*, 2000, **6**, 560.
44. D. Besser, R. Olender, R. Rosenfeld, O. Arad and S. Reismann, *J. Pept. Res.*, 2000, **56**, 337.
45. K. Gademann, M. Ernst, D. Seebach and D. Hoyer, *Helv. Chim. Acta*, 2000, **83**, 16.
46. P. W. Sutton, A. Bradley, J. Farras, P. Romea, F. Urpi and J. Vilarrasa, *Tetrahedron*, 2000, **56**, 7947.
47. K. Shiomi, N. Arai, Y. Iwai, A. Turberg, H. Kölbl and S. Omura, *Tetrahedron Lett.*, 2000, **41**, 2141.
48. K. Yoshikawa, S. Tao and S. Arihara, *J. Nat. Prod.*, 2000, **63**, 540.
49. X. Dai, Z. Su and J. O. Liu, *Tetrahedron Lett.*, 2000, **41**, 6295.
50. J. S. Davies, C. Enjalbal, C. Nguyen, L. Al-Jamri and C. Naumer, *J. Chem. Soc. Perkin Trans. 1*, 2000, 2907.
51. F. Peri, L. Cipolla, B. La Ferla and F. Nicotra, *Chem. Commun.* (*Cambridge*), 2000, 2303.
52. G. V. Nikiforovich, K. E. Köver, W.-J. Zhang and G. R. Marshall, *J. Am. Chem. Soc.*, 2000, **122**, 3262.
53. F. Schumann, A. Mueller, M. Koksch, G. Mueller and N. Sewald, *J. Am. Chem. Soc.*, 2000, **122**, 12009.
54. C. Baysal and H. Meirovitch, *Biopolymers*, 2000, **53**, 423.
55. Y.-C. Tang, X.-M. Gao, G.-L. Tian and Y.-H. Ye, *Chem. Lett.*, 2000, 826.
56. M. Doi, T. Ishida, Y. Katsuya, Y. Mezaki, M. Sasaki, A. Terashima, T. Taniguchi, H. Hasegawa and M. Shiono, *Chem. Commun.* (*Cambridge*), 2000, 743.
57. L. C. M. Ngoka and M. L. Gross, *J. Mass Spectrom.*, 2000, **35**, 265.
58. A. Qureshi, P. L. Colin and J. D. Faulkner, *Tetrahedron*, 2000, **56**, 3679.
59. I. Erdogan, J. Tanaka and T. Higa, *FABAD Farm. Bilimer Der.*, 2000, **25**, 7.
60. Y. Hitotsuyanagi, T. Aihara and K. Takeya, *Tetrahedron Lett.*, 2000, **41**, 6127.
61. Y. Hitotsuyanagi, T. Hasuda, Y. Matsumoto, H. Itokawa, K. Takeya and K. Yamaguchi, *Chem. Commun.* (*Cambridge*), 2000, 1633.
62. R.-H. Mattern, S. B. Moore, T.-A. Tran, J. K. Rueter and M. Goodman, *Tetrahed-*

ron, 2000, **56**, 9819.
63. A. S. Dutta, J. J. Gormley, M. Coath, L. Hassall, C. F. Hayward, P. R. Gellert, R. S. Kittlety, P. J. Alcock, R. Ferguson, T. Halterman, A. Jamieson, J. A. Moors, J. M. Moores, A. Rees, L. J. Wood, C. F. Reilly and D. Haworth, *J. Pept. Sci.*, 2000, **6**, 560.
64. L. L. Klein, L. Li, H.-J. Chen, C. B. Curty, D. A. DeGeoy, D. J. Grampovnik, C. L. Leone, S. A. Thomas, C. M. Yeung, K. W. Funk, V. Kishore, E. O. Lundell, D. Wodka, J. A. Meulbroek, J. D. Alder, A. M. Nilius, P. A. Lartey and J. J. Plattner, *Bioorg. Med. Chem.*, 2000, **8**, 1677.
65. D. B. Smithrud, P. A. Benkovic, S. J. Benkovic, V. Roberts, J. Liu, I. Neagu, S. Iwama, B. W. Phillips, A. B. Smith and R. Hirschmann, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 1953.
66. S. Kubik and R. Goddard, *Chem. Commun.* (*Cambridge*), 2000, 633.
67. J. Wang, S. Osada, H. Kodama and M. Kondo, *Bull. Chem. Soc. Jpn.*, 2000, **73**, 1221.
68. C. Baraguey, A. Blond, I. Correia, J.-L. Pousset, B. Bodo and C. Auvin-Guette, *Tetrahedron Lett.*, 2000, **41**, 325.
69. G. R. Pettit, B. E. Toki and J.-P. Xu and D. C. Brune, *J. Nat. Prod.*, 2000, **63**, 22.
70. C. Baysal and H. Meirovitch, *Biopolymers*, 2000, **54**, 416.
71. Z.-m. Yang, W.-y. Xin and J. Zhang, *Yunnan Daxue Xuebao Ziran Kexueban*, 2000, **22**, 123.
72. Z. Ding, J. Zhou, N. Tan and R. Teng, *Planta Med.*, 2000, **66**, 386.
73. Y. X. Cheng, J. Zhou, N. H. Tan and Z. T. Ding, *Chin. Chem. Lett.*, 2000, **11**, 595.
74. Z. Ding, J. Zhou, Y. X. Cheng and N. H. Tan, *Chin. Chem. Lett.*, 2000, **11**, 593.
75. Z. Ding, Y. Wang, J. Zhou, N. Tan and H. Wu, *Yunnan Zhiwu Yanjiu*, 2000, **22**, 331.
76. D. L. Boger and J. K. Lee, *J. Org. Chem.*, 2000, **65**, 5996.
77. M. Saviano, C. Isernia, F. Rossi, B. DiBlasio, R. Iacovino, M. Mazzeo, C. Pedone and E. Benedetti, *Biopolymers*, 2000, **53**, 189.
78. R. M. Cusack, L. Grondahl, G. Abbenante, D. P. Fairlie, L. R. Gahan, G. R. Hanson and T. W. Hambley, *J. Chem. Soc. Perkin Trans. 2*, 2000, 323.
79. J. Sanchez-Queseda, M. R. Ghadiri, H. Bayley and O. Braha, *J. Am. Chem. Soc.*, 2000, **122**, 11757.
80. M. C. Roy, I. I. Otani, T. Ichiba, J. Tanaka, R. Satari and T. Higa, *Tetrahedron*, 2000, **56**, 9079.
81. Y. F. Hallock, R. C. Sowder II, L. K. Pannell, C. B. Hughes, D. G. Johnson, R. Gulakowski, J. H. Cardellina II and M. R. Boyd, *J. Org. Chem.*, 2000, **65**, 124.
82. K. R. Gustafson, L. K. Walton, R. C. Sowder II, D. G. Johnson, L. K. Pannell, J. H. Cardellina II and M. R. Boyd, *J. Nat. Prod.*, 2000, **63**, 176.
83. F. Hubler, T. Rückle, L. Patiny, T. Muamba, J. F. Guichou, M. Mutter and R. Wenger, *Tetrahedron Lett.*, 2000, **41**, 7193.
84. P. Li and J. C. Xu, *J. Org. Chem.*, 2000, **65**, 2951.
85. A. Jegerov, L. Cvak, A. Husek, P. Simek, A. Heydová, J. Ondrácek, S. Pakhomova, M. Hušák, B. Kratochvil, P. Sedmera and V. Havlicek, *Collect. Czech. Chem. Commun.*, 2000, **65**, 1317.
86. M. Hušák, B. Kratochvil, I. Cisarová and A. Jegorov, *Collect. Czech. Chem. Commun.*, 2000, **65**, 1950.
87. A. Jegorov, S. Pakhomova, M. Hušák, B. Kratochvil, Z. Zak, L. Cvak and M. Buchta, *J. Inclusion Phenom. Macrocyclic Chem.*, 2000, **37**, 137.
88. R. Dieden, D. Latinne, C. Baldari, N. Maton, A. Aubry, R. K. Verbeek, M. Zurini and G. J. J. Lhoest, *Spectroscopy* (*Amsterdam, Neth.*), 2000, **14**, 215.
89. V. Havlicek, A. Jegerov and P. Sedmera, *New Adv. Anal. Chem.*, Atta-ur-Rahman

ed., Harwood Acad. Publ., Amsterdam, 2000, **P2**, 339–368.
90. S. Rana, J. Dubuc, M. Bradley and P. White, *Tetrahedron Lett*., 2000, **41**, 5135.
91. M. Cudic, J. D. Wade and L. Otvos Jr., *Tetrahedron Lett*., 2000, **41**, 4527.
92. R. Oliva, L. Falcigno, G. D'Auria, M. Saviano, L. Paolillo, G. Ansanelli and G. Zanotti, *Biopolymers*, 2000, **53**, 581.
93. L. Jiang, K. Moehle, B. Dhanapal, D. Obrecht and J. A. Robinson, *Helv. Chim. Acta*, 2000, **83**, 3097.
94. C. McInnes, L. H. Kondejewski, R. S. Hodges and B. D. Sykes, *J. Biol. Chem*., 2000, **275**, 14287.
95. M. Takeuchi, T. Taniguchi and K. Ogasawara, *Tetrahedron*: *Asymmetry*, 2000, **11**, 1601.
96. S. S. Mitchell, D. J. Faulkner, K. Rubins and F. D. Bushman, *J. Nat. Prod*., 2000, **63**, 279.
97. H. Luesch, W. Y. Yoshida, R. E. Moore, V. J. Paul and S. L. Mooberry, *J. Nat. Prod*., 2000, **63**, 611.
98. M. A. Rashid, K. R. Gustafson, J. L. Boswell and M. R. Boyd, *J. Nat. Prod*., 2000, **63**, 956.
99. L. T. Tan, R. T. Williamson, W. H. Gerwick, K. S. Watts, K. McGough and R. Jacobs, *J. Org. Chem*., 2000, **65**, 419.
100. K. Ishida, H. Nakagawa and M. Murakami, *J. Nat. Prod*., 2000, **63**, 1315.
101. L. A. Morris, J. J. Kettenes van den Bosch, K. Verslius, G. S. Thompson and M. Jaspars, *Tetrahedron*, 2000, **56**, 8345.
102. C. D. J. Boden and G. Pattenden, *J. Chem. Soc. Perkin Trans. 1*, 2000, 875.
103. P. Wipf and Y. Uto, *J. Org. Chem*., 2000, **65**, 1037.
104. C. D. J. Boden, M. Norley and G. Pattenden, *J. Chem. Soc. Perkin Trans. 1*, 2000, 883.
105. A. Bertram and G. Pattenden, *Synlett*, 2000, 1519; A. J. Blake, J. S. Hannan, K. A. Jolliffe and G. Pattenden, *Synlett*, 2000, 1515.
106. C. Boss, P. H. Rasmussen, A. R. Wartini and S. R. Waldvogel, *Tetrahedron Lett*., 2000, **41**, 6327.
107. G. Haberhauer, L. Somogyi and J. Rebek Jr., *Tetrahedron Lett*., 2000, **41**, 5013.
108. M. C. Bagley, K. E. Bashford, C. L. Hesketh and C. J. Moody, *J. Am. Chem. Soc*., 2000, **122**, 3301.
109. P. Wipf, P. C. Miller and C. M. Grant, *Tetrahedron*, 2000, **56**, 9143.
110. C. A. Dvorak, W. D. Schmitz, D. J. Poon, D. C. Pryde, J. P. Lawson, R. A. Amos and A. I. Meyers, *Angew. Chem. Int. Ed*., 2000, **39**, 1664.
111. M. C. Bagley, S. L. Hind and C. J. Moody, *Tetrahedon Lett*., 2000, **41**, 6879; M. C. Bagley, C. J. Moody and A. G. Pepper, *Tetrahedron Lett*., 2000, **41**, 6901; F. Lach and C. J. Moody, *Tetrahedron Lett*., 2000, **41**, 6893.
112. X. Chen, L. Esser and P. G. Harran, *Angew. Chem. Int. Ed*., 2000, **39**, 937.
113. K. C. Nicolaou, S. A. Snyder, K. B. Simonsen and A. E. Koumbis, *Angew. Chem. Int. Ed*., 2000, **39**, 3473.
114. M. Sitachitta, R. T. Williamson and W. H. Gerwick, *J. Nat. Prod*., 2000, **63**, 197.
115. F. D. Horgen, W. Y. Yoshida and P. J. Scheuer, *J. Nat. Prod*., 2000, **63**, 461.
116. H. Vervoort, W. Fenical and R. De A. Epifanio, *J. Org. Chem*., 2000, **65**, 782.
117. K. Fujii, K. Sivonen, E. Naganawa and K-I. Harada, *Tetrahedron*, 2000, **56**, 725.
118. F. D. Horgen, D. B. delos Santos, G. Goetz, B. Sakamoto, Y. Kan, H. Nagai and P. J. Acheuer, *J. Nat. Prod*., 2000, **63**, 152.
119. J.-F. Hu, D. Wunderlich, I. Sattler, X.-Z. Feng, S. Grabley and R. Thiericke, *Eur. J. Org. Chem*., 2000, 3353.

120. K. Umezawa, Y. Ikeda, Y. Uchihata, H. Naganawa and S. Kondo, *J. Org. Chem.*, 2000, **65**, 459.
121. Y. Hatout, Y-P. Ho, V. Ryzhov, P. Demirev and C. Fenselau, *J. Nat. Prod.*, 2000, **63**, 1492.
122. M. Chu, T. M. Chan, P. Das, R. Mierzawa, M. Patel and M. S. Puar, *J. Antibiotics*, 2000, **53**, 736.
123. D. Ishiyma, T. Sato, R. Honda, H. Senda, H. Konno and S. Kanazawa, *J. Antibiotics*, 2000, **53**, 525.
124. P. Jeschke, W. Etzel, A. Harder, M. Schindler, A. Gohrt, U. Pleiss, H. Kleinkauf, R. Zocher, G. Thielking, W. Gau and G. Bonse, *Bioorg. Chem.*, U. Diederischen ed., Wiley-VCH, Verlag GmbH, Weinheim, 1999, p. 207.
125. M. Eggen, C. J. Mossman, S. B. Buck, S. K. Nair, L. Bhat, S. M. Ali, E. A. Reiff, T. C. Boge and G. I. Georg, *J. Org. Chem.*, 2000, **65**, 7792.
126. A. K. Gosh and A. Bischoff, *Org. Lett.*, 2000, **2**, 1573.
127. N. Murakami, W. Wang, N. Ohyabu, T. Ito, S. Tamura, S. Aoki, M. Kobayashi and I. Kitagawa, *Tetrahedron*, 2000, **56**, 9121.
128. N. Murakami, W. Wang, S. Tamura and M. Kobayashi, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1823.
129. R. A. Barrow, R. E. Moore, L.-H. Li and M. A. Tius, *Tetrahedron*, 2000, **56**, 3339.
130. J. A. Christopher, P. J. Kocienski, A. Kuhl and R. Bell, *Synlett*, 2000, 463.
131. J. Liang, E. D. Moher, R. E. Moore and D. W. Hoard, *J. Org. Chem.*, 2000, **65**, 3143.
132. P. Portonovo, X. Ding, M. S. Leonard and M. M. Joullie, *Tetrahedron*, 2000, **56**, 3687.
133. M. D. Vera, A. J. Pfizenmayer, X. Ding, D. Xiao and M. M. Joullie, *Bioorg. Med. Chem. Lett.*, 2000, **11**, 13.
134. B. Liang, M. D. Vera and M. M. Joullie, *J. Org. Chem.*, 2000, **65**, 4762.
135. M. M. Joullie, P. Portonovo, B. Liang and D. J. Richard, *Tetrahedron Lett.*, 2000, **41**, 9373.
136. K. C. Nicolaou, F. Murphy, S. Borluenga, T. Ohshima, H. Wei, J. Xu, D. L. F. Gray and O. Baudoin, *J. Am. Chem. Soc.*, 2000, **122**, 3830.
137. P. Hall, J. Brun, R. Denni and R. Metternich, *Synlett*, 2000, 315.
138. Y.-Z. Zhang, X. Sun, D. J. Zeckner, R. K. Sachs, W. L. Current and S.-H. Chen, *Bioorg. Med. Chem. Lett.*, 2000, **11**, 123.
139. C. Hermann, G. C. G. Pais, A. Geyer, S. M. Kuhnert and M. E. Maier, *Tetrahedron*, 2000, **56**, 8461.
140. A. Lopez-Macia, J. C. Jimenez, M. Royo, E. Giralt and F. Albericio, *Tetrahedron Lett.*, 2000, **41**, 9765.
141. M. A. Ciufolini, D. Valognes and N. Xi, *J. Het. Chem.*, 1999, **36**, 1409.
142. M. A. Ciufolini, D. Valognes and N. Xi, *Angew. Chem. Int. Ed.*, 2000, **39**, 2493.
143. D. L. Boger, Y. Chen and C. A. Foster, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1741.
144. H. H. Wasserman, J.-H. Chen and M. Xia, *Helv. Chim. Acta*, 2000, **83**, 2607.
145. H. Kogen, T. Kiho, M. Nakayama, Y. Furukawa, T. Kinoshita and M. Inukai, *J. Am. Chem. Soc.*, 2000, **122**, 10214.
146. K. N. Koch, A. Linden, and H. Heimgartner, *Helv. Chim. Acta*, 2000, **83**, 233.
147. K. N. Koch and H. Heimgartner, *Helv. Chim. Acta*, 2000, **83**, 1881.
148. I. Nakanishi, A. Fujikawa, K. Imai and A. Sato, *J. Pept. Res.*, 2000, **55**, 120.
149. P. D. White in *Fmoc Solid Phase Pept. Synth.*, W. C. Chan and P. D. White eds., Oxford University Press, 2000, p. 183.
150. N. Kuder, T. Zelinski, T. Pathak, O. Seitz and H. Waldmann, *Bioorg. Med. Chem.*, 2000, **8**, 2433.

151. Z. Kupihar, G. Varadi, E. Monostori and G. K. Toth, *Tetrahedron Lett*., 2000, **41**, 4457.
152. K. Miyoshi, A. Otaka, M. Kaneko, H. Tamamura and N. Fujii, *Chem. Pharm. Bull*., 2000, **48**, 1230.
153. J. Sander and H. Waldmann, *Chem-Eur. J*., 2000, **6**, 1564.
154. T. Kappes-Roth and H. Waldmann, *J. Chem. Soc. Perkin Trans. 1*, 2000, 2579.
155. T. Kawakami, K. Hasegawa and S. Aimoto, *Bull. Chem. Soc. Jpn*., 2000, **73**, 197.
156. G. S. Cockerill, H. J. Easterfield, J. M. Percy and S. Pintat, *J. Chem. Soc. Perkin Trans. 1*, 2000, 2591.
157. T. R. Burke, D.-G. Liu and Y. Gao, *J. Org. Chem*., 2000, **65**, 6288.
158. P. Furet, G. Caravatti, A. A. Denholm, A. Faessler, H. Fretz, C. Garcia-Echeveria, B. Gay, E. Irving, N. J. Press, J. Rahuel, J. Schoepfer and C. V. Walker, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2337.
159. C. V. Walker, G. Caravatti, A. A. Denholm, J. Egerton, A. Faessler, P. Furet, C. Garcia-Echeverria, B. Gay, E. Irving, K. Jones, A. Lambert, N. J. Press and J. Woods, *Bioorg. Med. Chem. Lett*., 2000, **10**, 2343.
160. F. Gasparini, W. Inderbitzin, E. Francotte, G. Lecis, P. Richert, Z. Dragic, R. Kuhn and P. J. Flor, *Bioorg. Med. Chem. Lett*., 2000, **10**, 1241.
161. M. Cudic, P. Bulet and L. Otvos Jr., *Protein Pept. Lett*., 2000, **7**, 143.
162. H. Tokuyama and T. Fukuyama, *Kagaku* (*Kyoto*), 2000, **55**, 68.
163. J. Zhu, *Methods Mol. Med*., 1999, **23**, 293.
164. M. Bois-Choussy, C. Vergne, L. Neuville and J. Zhu, *Actual. Chim*., 2000, **7**/**8**, 66.
165. D. L. Boger, S. H. Kim, S. Miyazaki, H. Strittmatter, J.-H. Weng, Y. Mori, O. Rogel, S. L. Castle and J. J. McAtee, *J. Am. Chem. Soc*., 2000, **122**, 7416.
166. D. L. Boger, J.-H. Weng, S. Miyazaki, J. J. McAtee, S. L. Castle, S. H. Kim, Y. Mori, O. Rogel, H. Strittmatter and Q. Jin, *J. Am. Chem. Soc*., 2000, **122**, 10047.
167. A. J. Pearson and P. O. Belmont, *Tetrahedron Lett*., 2000, **41**, 1671.
168. K. C. Nicolaou, R. Hughes, S. Y. Cho, N. Winssinger, C. Smethurst, H. Labischinski and R. Endermann, *Angew. Chem. Int. Ed*., 2000, **39**, 3823.
169. K. C. Nicolaou, N. Winssinger, R. Hughes, C. Smethurst and S. Y. Cho, *Angew. Chem. Int. Ed*., 2000, **39**, 1084.
170. E. Freund, F. Vitali, A. Linden and J. A. Robinson, *Helv. Chim. Acta*, 2000, **83**, 2572.
171. L. Neuville, M. Bois-Choussy and J. Zhu, *Tetrahedron Lett*., 2000, **41**, 1747.
172. I. Malnar and C. J. Sih, *Tetrahedron Lett*., 2000, **41**, 1907.
173. R. J. Pieters, *Tetrahedron Lett*., 2000, **41**, 7541.
174. L. Frish, F. Sansone, A. Casnati, R. Ungaro and Y. Cohen, *J. Org. Chem*., 2000, **65**, 5026.
175. J. Rao, J. Lahiri, R. M. Weis and G. M. Whitesides, *J. Am. Chem. Soc*., 2000, **122**, 2698.
176. A. J. Pearson and J.-N. Heo, *Tetrahedron Lett*., 2000, **41**, 5991.
177. O. W. Choroba, D. H. Williams and J. B. Spencer, *J. Am. Chem. Soc*., 2000, **122**, 5389.
178. D. P. O'Brien, P. N. Kirkpatrick, S. W. O'Brien, T. Staroske, T. I. Richardson, D. A. Evans, A. Hopkinson, J. B. Spencer and D. H. Williams, *Chem. Commun*. (*Cambridge*), 2000, 103.
179. C.-H. Lee, H. Kyung and Y. Lim, *J. Microbiol. Biotech*., 2000, **10**, 733.
180. M. Schlauch and A. W. Frahm, *J. Chromatogr. A*., 2000, **868**, 197.
181. A. Berthod, X. Chen, J. P. Kullman, D. W. Armstrong, F. Gasparrini, I. D'Acquarica, C. Villani and A. Carotti, *Anal. Chem*., 2000, **72**, 1767.
182. R. Kerns, S. D. Dong, S. Fukuzawa, J. Carbeck, J. Kohler, L. Silver and D. Kahne,

J. Am. Chem. Soc., 2000, **122**, 12608.
183. O. A. Mirgorodskaya, E. N. Osufyeva, D. E. Kolume, T. J. D. Joergensen, P. Roepstorff, A. Yu. Pavlov, O. V. Miroshnikova and M. N. Preobrazhenskaya, *Russ. J. Bioorg. Chem.*, 2000, **26**, 566.
184. F. Neese, J. M. Zaleski, K. L. Zaleski and E. I. Solomon, *J. Am. Chem. Soc.*, 2000, **122**, 11703.
185. H. Herzner, T. Reipen, M. Schultz and H. Kunz, *Chem. Rev.*, 2000, **100**, 4495.
186. H. Kunz, B. Lohr and J. Habermann in '*Carbohydrates*', P. Finch ed., Kluwer Publ., Netherlands, 1999, p. 187.
187. H. Kunz and M. Schultz in *Carbohydr. Chem. Biol.*, B. Ernst, G. W. Hart and P. Sinay eds., Wiley-VCH Verlag, Germany, 2000, **1**, 267.
188. H. P. Wessel in *Carbohydr. Chem. Biol.*, B. Ernst, G. W. Hart and P. Sinay eds., Wiley-VCH Verlag, Germany, 2000, **1**, 565.
189. J. Kihlberg in *Fmoc Solid Phase Pept. Synth.*, W. C. Chan and P. D. White eds., Oxford University Press, 2000, p. 195.
190. H. Hojo and Y. Nakahara, *Curr. Protein Sci.*, 2000, **1**, 23.
191. P. M. St. Hilaire and M. Meldal, *Angew. Chem. Int. Ed.*, 2000, **39**, 1163.
192. I. G. Smirnova, G. S. Katrukha and I. V. Denisova, *Vestn. Mosk. Univ. Ser* 2: *Khim.*, 2000, **41**, 75.
193. G. Liu, S. D. Zhang, S.-Q. Xia, and Z.-K. Ding, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1361.
194. I. Shin and J. Lee, *Synlett*, 2000, 1297.
195. C. Kieburg, K. Sadalapure and T. K. Lindhorst, *Eur. J. Org. Chem.*, 2000, 2035.
196. R. J. Tennant-Eyles, B. G. Davis and A. J. Fairbanks, *Tetrahedron*: *Asymmetry*, 2000, **11**, 231.
197. B. Holm, J. Broddefalk, S. Flodell, E. Wellner and J. Kihlberg, *Tetrahedron*, 2000, **56**, 1579.
198. G. Dudziak, N. Bezay, T. Schwientek, H. Clausen, H. Kunz and A. Liese, *Tetrahedron*, 2000, **56**, 5865.
199. W.-T. Jiaang, M.-Y. Chang, P.-H. Tseng and S.-T. Chen, *Tetrahedron Lett.*, 2000, **41**, 3127.
200. K. M. Halkas, P. M. St. Hilaire, A. M. Jansson, C. H. Gotfredsen and M. Meldal, *J. Chem. Soc. Perkin Trans. 1*, 2000, 2127.
201. W.-T. Jiaang, P.-H. Tseng and S.-T. Chen, *Synlett*, 2000, 797.
202. J. R. Allen and S. J. Danishefsky, *J. Prakt. Chem.*, 2000, **342**, 736.
203. S. Ando, Y. Nakahara, Y. Ito, T. Ogawa and Y. Nakahara, *Carbohydr. Res.*, 2000, **329**, 773.
204. P. W. Glunz, S. Hintermann, L. J. Williams, J. B. Schwarz, S. D. Kuduk, V. Kudryashov, K. O. Lloyd and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2000, **122**, 7273.
205. K. Kurokawa, H. Kumihara and H. Kondo, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1827.
206. A. Ishii, H. Hojo, A. Kobayashi, K. Nakamura, Y. Nakahara, Y. Ito and Y. Nakahara, *Tetrahedron*, 2000, **56**, 6235.
207. S. Komba, M. Meldal, O. Werdelin and T. Jensen, *J. Peptide Sci.*, 2000, **6**, 585.
208. L. Chen, K. J. Jensen, J. Tejbrant, J. E. Taylor, B. A. Morgan and G. Barany, *J. Pept. Res.*, 2000, **55**, 81.
209. S. Vichier-Guerre, R. Lo-Man, S. Bay, E. Denaud, H. Nakada, C. Leclerc and D. Cantacuzène, *J. Pept. Res.*, 2000, **55**, 173.
210. Yu. Nakahara, S. Ando, M. Itakura, N. Kumabe, H. Hojo, Y. Ito and Yo. Nakahara, *Tetrahedron Lett.*, 2000, **41**, 6489.

211. K. Ajisaka and M. Miyasato, *Biosci. Biotechnol. Biochem.*, 2000, **64**, 1743.
212. K. M. Koeller, M. E. B. Smith, C.-H. Wong, *Bioorg. Med. Chem.*, 2000, **8**, 1017.
213. K. M. Koeller, M. E. B. Smith, R. F. Huang and C.-H. Wong, *J. Am. Chem. Soc.*, 2000, **122**, 4241.
214. P. Sjolin and J. Kihlberg, *Tetrahedron Lett.*, 2000, **41**, 4435.
215. M. C. Lo, H. Men, A. Branstrom, J. Helm, N. Yao, R. Goldman and S. Walker, *J. Am. Chem. Soc.*, 2000, **122**, 3540.
216. E. J. Bilsky, R. D. Egleton, S. A. Mitchell, M. M. Palian, P. Davis, J. D. Huber, H. Jones, H. I. Yamamura, J. Janders, T. P. Davis, F. Porreca, V. J. Hruby and R. Polt, *J. Med. Chem.*, 2000, **43**, 2586.
217. C. T. Kriss, B.-S. Lou, L. Z. Szabo, S. A. Mitchell, V. J. Hruby and R. Polt, *Tetrahedron: Asymmetry*, 2000, **11**, 9.
218. N. B. Malkar, J. L. Lauer-Fields and G. B. Fields, *Tetrahedron Lett.*, 2000, **41**, 1137.
219. Y. Ito, M. Gerz and Y. Nakahara, *Tetrahedron Lett.*, 2000, **41**, 1039.
220. K. Peilstöcker and H. Kunz, *Synlett*, 2000, 820.
221. K. Peilstöcker and H. Kunz, *Synlett*, 2000, 823.
222. P. Allef and H. Kunz, *Tetrahedron:Asymmetry*, 2000, **11**, 375.
223. Zh.-G. Wang, X. Zhang, D. Live and S. J. Danishefsky, *Angew. Chem. Int. Ed.*, 2000, **39**, 3652.
224. Y. Nishiyama, T. Yoshikawa, N. Ohara, K. Kurita, K. Hojo, H. Kamada, Y. Tsutsumi, T. Mayumi and K. Kawasaki, *J. Chem. Soc. Perkin Trans. 1*, 2000, 1161.
225. Y. Ohnishi, H. Ando, T. Kawai, Y. Nakahara and Y. Ito, *Carbohydr. Res.*, 2000, **328**, 263.
226. L. Jobron and G. Hummel, *Angew. Chem. Int. Ed.*, 2000, **39**, 1621.
227. J. P. Malkinson, R. A. Falconer and I. Toth, *J. Org. Chem.*, 2000, **65**, 5249.
228. D. Bailey, D. V. Renouf, D. G. Large, C. D. Warren and E. F. Hounsell, *Carbohydr. Res.*, 2000, **324**, 242.
229. A. Dondoni and A. Marra, *Chem. Rev.*, 2000, **100**, 4395.
230. A. Dondoni, G. Mariotti and A. Marra, *Tetrahedron Lett.*, 2000, **41**, 3483.
231. S. P. Vincent, A. Schleyer and C.-H. Wong, *J. Org. Chem.*, 2000, **65**, 4440.
232. E. Wellner, T. Gustafsson, J. Backlund, R. Holmdahl and J. Kihlberg, *ChemBioChem.*, 2000, **1**, 272.
233. M. Tagashira, T. Kitagawa, N. Nozato, S. Isonishi, A. Okamato, K. Ochiai and Y. Ohtake, *Chem. Pharm. Bull.*, 2000, **48**, 575.
234. C.-Y. Tsai, X. Huang and C.-H. Wong, *Tetrahedron Lett.*, 2000, **41**, 9499.
235. V. Wittmann and S. Seeberger, *Angew. Chem. Int. Ed.*, 2000, **39**, 4348.
236. D. Ramos, P. Rollin and W. Klaffe, *Angew. Chem. Int. Ed.*, 2000, **39**, 396.
237. W. T. Collard, D. L. Evers, D. L. McKenzie and K. G. Rice, *Carbohydr. Res.*, 2000, **323**, 176.
238. Y. Ohnishi, M. Ichikawa and Y. Ichikawa, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1289.
239. L. Jobron and G. Hummel, *Org. Lett.*, 2000, **2**, 2265.
240. H. Luesch, W. Y. Yoshida, R. E. Moore and V. J. Paul, *J. Nat. Prod.*, 2000, **63**, 1106.
241. W. Aretz, J. Meiwes, G. Seibert, G. Vobis and J. Wink, *J. Antibiotics*, 2000, **53**, 807; L. Vertesy, E. Ehlers, H. Kogler, M. Kurz, J. Meiwes, G. Seibert, M. Vogel and P. Hammann, *J. Antibiotics.*, 2000, **53**, 816.
242. D. Kadeireit and H. Waldmann, *Monatsh. Chem.*, 2000, **131**, 571.
243. E. K. Dolence, J. M. Dolence and C. D. Poulter, *J. Comb. Chem.*, 2000, **2**, 522.
244. F. Yokokowa, H. Fujiwara and T. Shioiri, *Tetrahedron*, 2000, **56**, 1759.
245. R. Machauer and H. Waldmann, *Angew. Chem. Int. Ed.*, 2000, **39**, 1449.
246. D. Bonnet, L. Bourel, H. Gras-Masse and O. Melnyk, *Tetrahedron Lett.*, 2000, **41**,

10003.
247. E. Medina, A. Moyano, M. A. Pericas and A. Riera, *Helv. Chim. Acta*, 2000, **83**, 972.
248. K. Uchida, Y. Nishiyama, N. Yokota and H. Yamaguchi, *J. Antibiotics*, 2000, **53**, 1175.
249. J. Khono, Y. Koguchi, M. Nishio, K. Nakao, M. Kuroda, R. Shimizu, T. Ohnuki and S. Komatsubara, *J. Org. Chem*., 2000, **65**, 990.
250. H.-y. Lin, C.-H. Chen, B.-J. You, K. C. S. Liu and S.-S. Lee, *J. Nat. Prod*., 2000, **63**, 1338.
251. T. Laïb, M. Bois-Choussy and J. Zhu, *Tetrahedron Lett*., 2000, **41**, 7645.
252. V. Jullian, E. Shepherd, T. Gelbrich, M. B. Hursthouse and J. D. Kilburn, *Tetrahedron Lett*., 2000, **41**, 3963.
253. P. D. Henley, C. P. Waymark, I. Gillies and J. D. Kilburn, *J. Chem. Soc. Perkin Trans. 1*, 2000, 1021.
254. H. Zhao and W. Hua, *J. Org. Chem*., 2000, **65**, 2933.
255. J. P. Tam, Y.-A. Lu and J.-L. Yang, *Biochemistry*, 2000, **39**, 7159.
256. D. Ranganathan, V. Haridas, R. Nagaraj and I. L. Karle, *J. Org. Chem*., 2000, **65**, 4415.
257. M. Planas, E. Bordaji and G. Barany, *Tetrahedron Lett*., 2000, **41**, 4097.
258. J. C. Verheijen, G. M. Grotenbreg, L. H. de Ruyter, P. A. M. Van der Klein, G. A. Van der Marel and J. H. Van Boom, *Tetrahedron Lett*., 2000, **41**, 3991.
259. T. Laïb and J. Zhu, *Synlett*, 2000, 1363.
260. S. Reyes, M. Pattarawarapan, S. Roy and K. Burgess, *Tetrahedron*, 2000, **56**, 9809.
261. D. Raganathan, C. Lakshmi, V. Haridas and M. Gopikumar, *Pure Appl. Chem*., 2000, **72**, 365.
262. J. M. Dougherty, D. A. Probst, R. E. Robinson, J. D. Moore, T. A. Klein, K. A. Snelgrove and P. R. Hanson, *Tetrahedron*, 2000, **56**, 9781.
263. B. Wang, S. Gangwar, G. Pauletti, T. Siahaan and R. T. Borchardt, *Methods Mol. Med*., 1999, **23**, 53.
264. S. Gangwar, G. M. Pauletti, T. J. Siahaan, V. J. Stella and R. T. Borchardt, *Methods, Mol. Med*., 1999, **23**, 37.

5
Metal Complexes of Amino Acids and Peptides

BY E. FARKAS AND I. SÓVÁGÓ

1 Introduction

This chapter deals with the most important results published on the synthesis, structures, solution equilibria, reactivity and applications of the metal complexes of amino acids, peptides and related ligands in 1999 and 2000. The major source of the articles reported here is CA Selects on Amino Acids, Peptides and Proteins,[1] but the title pages of the most common journals of inorganic, bioinorganic and coordination chemistry have also been surveyed. In addition to the hardcopies of the above mentioned journals the abstracts reported by some electronic databases (*e.g.* Web of Science Citation Databases, Institute for Scientific Information) have also been searched for the subject 'metal complexes of amino acids and peptides'. Of course, the number of hits matching the queries was tremendously high. Consequently, only those papers which focus on the complexation reactions of amino acids and peptides are included in this review.

As in the previous volumes, the results published on the metal complexes of amino acids (Section 2) and peptides (Section 3) are treated separately, although there are many publications dealing with the coordination chemistry of both groups of ligands. In addition to the naturally occurring amino acids some synthetic analogues and derivatives of amino acids and peptides were also considered if they have biological or theoretical significance or important applications in chemistry or medicine. The number of papers dealing with the synthetic, analytical or biomedical applications of amino acid and peptide complexes has significantly increased in the past few years. Thus, the papers were classified into four sub-sections in both sections: (a) synthesis and structural studies, (b) solution equilibria, (c) reactivity and kinetics and (d) synthetic, analytical and biomedical applications.

The most recent reviews published in 1999 and 2000 cover some specific aspects of the coordination chemistry of amino acids, peptides and the biomedical applications of these complexes. The biological activity of cisplatin and related drugs received increasing attention in the past decade and resulted in a large number of publications including several reviews on the platinum(II) and palladium(II) complexes of peptides.[2–4] It has already been widely accepted that

Amino Acids, Peptides and Proteins, Volume 33

non-coordinating side chains do not have a significant impact on the coordination chemistry of simple peptides. There are, however, some specific amino acid sequences which result in the enhanced stability of metal complexes. The specific structure–stability relationship was reviewed recently and it covers the most important findings on the role of non-coordinating histidyl and prolyl residues.[5] Glutathione is one of the most abundant organic substances in the human body. The presence of the thiol group in glutathione results in outstanding metal binding ability and redox activity of the ligand and the most important complex formation and redox reactions of both reduced and oxidized glutathione were reviewed.[6] Another publication deals with the biological chemistry of vanadium with a special emphasis on the interactions with glutathione.[7] Phosphonic derivatives of amino acids and peptides are versatile ligands with promising biological applications. The metal complexes of these ligands, including also the open-chain and macrocyclic aminophosphonates and aminophosphinates, were surveyed recently.[8]

Another group of review articles covered some aspects of the metal ion coordination in peptide synthesis. These include overviews on the cobalt(II) promoted synthesis of small peptides,[9] the metal-mediated modifications of the side chains of amino acids[10] and the possible role of copper(II) in peptide synthesis, involving peptide formation under prebiotic conditions.[11]

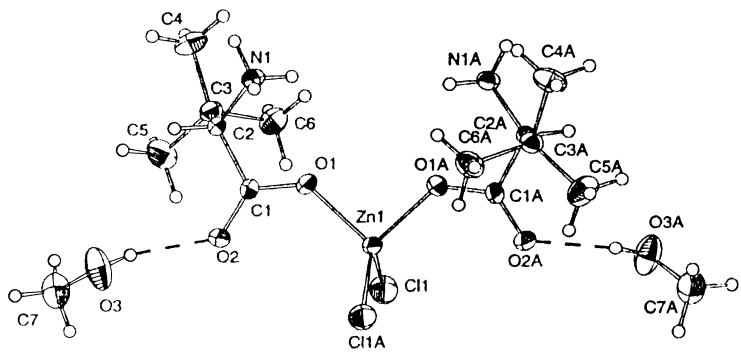
The better understanding of the structures and properties of zinc finger peptides and copper chaperons is among the most fascinating areas in bioinorganic chemistry and they have been briefly reviewed, recently.[12,13] The other review articles published in this field are mainly devoted to the biomedical applications of amino acids, and peptides and their metal complexes. Some therapeutic chelating agents are based upon simple amino acids and their biodegradability and applications are summarized by a series of related papers.[14] Bleomycins are glycopeptide antitumour antibiotics taking part in DNA binding and degradation, a process that is metal ion and oxygen dependent.[15] The use of metal complexes in clinical diagnostic imaging is increasing tremendously. These applications involve gamma scintigraphy, positron emission tomography (PET), magnetic resonance imaging (MRI) and metal-based radiopharmaceuticals. Metal complexes of peptides and related ligands are frequently used in these techniques and the most important applications have been reviewed.[16,17]

Nickel(II) compounds are known as human carcinogens and histones are likely targets for binding of nickel(II) ion in cells. The recent studies on the peptide and protein models on nickel(II) binding to histones have been reviewed.[18] The field of biomolecular architectures is a broad and interdisciplinary area of worldwide research that has been growing explosively. Novel synthetic approaches to the incorporation of metal complexes into nucleic acids and peptides have been described.[19]

2 Amino Acid Complexes

2.1 Synthesis and Structural Studies. – Numerous papers have been published on metal complexation of amino acids and derivatives during the past two years. Different spectroscopic, calorimetric, electrochemical and X-ray diffraction methods were used, and in many cases several methods were combined to characterize the complexes prepared. The main interest in transition metal complexes has remained, but the number of results for complexes of other metal ions such as alkali, alkaline earth metals and lanthanides is also increasing.

In several cases, studies on binary complexes of amino acids were carried out.[20–43] The structure of $Zn(L\text{-}Ala)_2$ was determined by X-ray crystallography. The Zn(II) ion is five-coordinated in the complex, two bidentate amino acidates and a carboxylate oxygen of another symmetry-related alaninate ion bind to the metal. However, distortion of the Zn site occurs when it is occupied by Cu(II) impurities.[22] Only monodentate carboxylate coordination of the zwitterionic form of L-*tert*-Leu was found in the biscomplex prepared from an aqueous solution of $ZnCl_2$ and L-*tert*-Leu (**1**).[23]



(1)

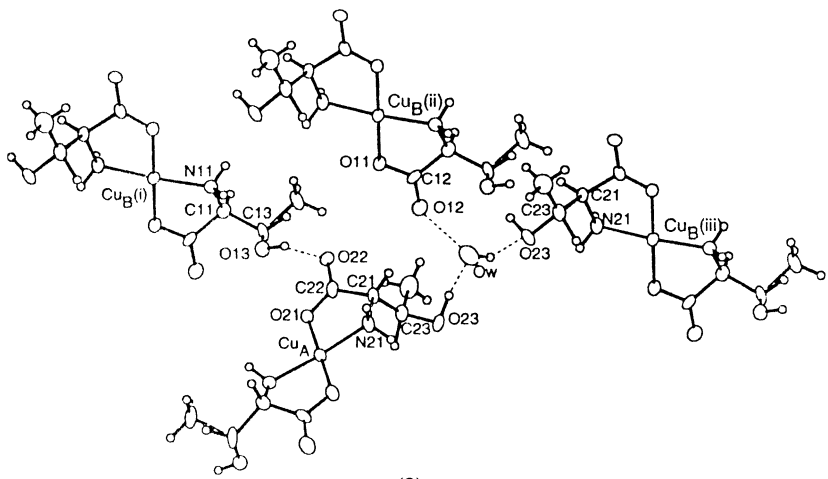
IR, thermogravimetry and differential scanning calorimetry were used to characterize the complexes $[CdCl_2.x\text{Gly}]$ ($x = 1, 2$). Usual bidentate coordination of Gly was found in the monocomplex, but only the carboxylates are coordinated in the biscomplex. The two chloride anions are also coordinated.[24] Alkyl moieties inserted into the side chain of amino acids increase the hydrophobic character of the molecules, but, at the same time, decrease their metal binding ability. Copper(II) complexes of a series of mono- and dialkylated L-alaninates were prepared as model compounds for superoxide dismutase (SOD)-mimetic activity. Both solid state and solution results confirmed that the alkylated derivatives form much less stable complexes than the parent copper(II)-L-alaninates.[25]

The influence of side chain coordination sites such as -OH, $-CONH_2$, -COOH and imidazole on the bonding mode and crystal structure of metal complexes have been discussed in several papers. The side chain -OH group of threonine generally does not play a significant role in the coordination. However, recent

results on the complex [Cu(L-Thr)$_2$]·H_2O revealed that -OH groups are involved in intermolecular H-bonds (**2**).[27]

Several results have been published for metal complexes of Asp, and the side chain carboxylate was generally found to be involved in the coordination.[28–30] As simple models for the active site of numerous metalloproteins, complexes of His were investigated in several laboratories during the period reviewed.[31–34] For example, its 1:1 complexes with Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) were studied by electrospray ionization mass spectrometry.[33] Due to its biological relevance, the coordination chemistry of peroxovanadates has a renewed interest. Three new oxodiperoxovanadate complexes formed with amino acids L-asparagine, L-phenylglycine and D,L-homocystine were synthesized in a recent work. All the complexes present one oxo ligand, two peroxo anions and one monodentate amino acid ligand in the inner coordination sphere of vanadium. The amino acid coordinates through one of its carboxylate oxygens. The stoichiometry of the complexes, however, is different. Mononuclear complexes are formed with L-asparagine and L-phenylglycine, but D,L-homocysteine bridges two oxodiperoxovanadium moieties through its carboxylate group. There is not any evidence of the coordination through the sulfur atom. Decomposition of the complexes by losing the peroxo ligands makes the V(V) oxidation state unstable and leads to the reduction of V(V) to V(IV).[35] Some new results appeared also for ruthenium–amino acid complexes.[36,37] According to these results, the α-amino acids Gly, Ala, Phe, Tyr and Leu form stable complexes with ruthenium, and in the presence of π-acid ligands such as triphenylphosphine (PPh_3), the +2 oxidation state of the metal becomes stabilized. Two bidentate amino acids and two PPh_3 are coordinated in the complexes formed.[36] Tridentate coordination of L-Met to Ru(IV) through the O atom of the carboxylate group, N atom of the amino group and the S atom was found in the ruthenium nitrosyl methionine complex [RuNO(Met)Cl_2].[37]

The complexation between lanthanide ions and amino acids was the subject of



(2)

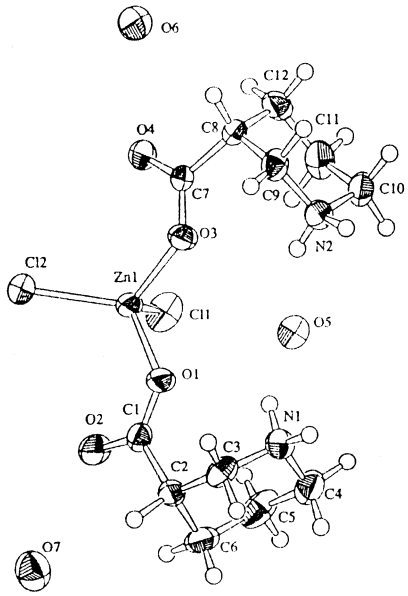
several papers.[38–42] Limited hydrolysis of lanthanide ions controlled by α-amino acids as supporting ligands resulted in the formation of mixed hydroxo clusters.[38] Interestingly, the lanthanum complex of Glu, $[La_2(Glu)_4(SO_4)_3]\cdot 8H_2O$ exhibits remarkable antitumour activity.[41] IR and ^{13}C NMR methods were used to determine the bonding mode in a new Eu(III) L-histidine complex, $[Eu(L\text{-}His)_3](ClO_4)_3$.[42] Some new complexes of histamine with nickel(II)[43] and copper(II)[44–46] were also prepared and characterized. Thiosulfate-assisted synthesis of [Cu(histamine)$_2$(SCN)](SCN) was achieved by adding histamine to the aqueous $CuCN/S_2O_3^{2-}$ reaction medium. Reaction with methionine produced [Cu(methioninato)$_2$]. On the contrary, when Cys was added to the reaction medium, [Cu(2-amino-2-thiazoline-carboxylate)$_2$] was obtained.[46]

Anthranilic acid (2-aba), a precursor to Trp, forms diverse complexes with alkaline earth metal ions. Four different complexes [Mg(2-aba)$_2$], $[Ca(2\text{-aba})_2(OH_2)_3]_\infty$, $[\{Sr(2\text{-aba})_2(OH_2)_2\}\cdot H_2O]_\infty$ and $[Ba(2\text{-aba})_2(OH_2)_2]_\infty$ have been prepared, and the structure of the three latter ones have been characterized by X-ray diffraction. While the calcium ions are heptacoordinated, the strontium and barium ions reveal a coordination number of 9. Moreover, the carboxylate groups present different chelating and bridging modes in the three complexes, and interestingly, apart from the carboxylate functionality, the amino group also binds to the metal centres in the case of strontium and barium complexes.[47]

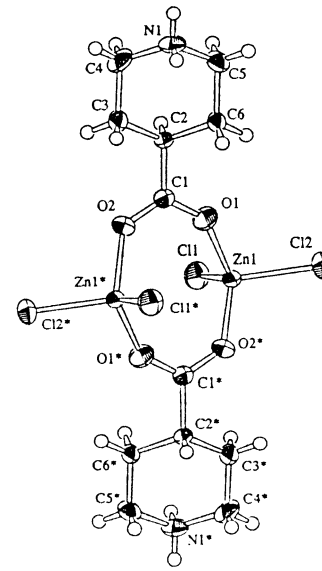
L-Piperidine-2-carboxylic acid is a non-proteinogenic amino acid and is a metabolite of the amino acid Lys. Zn(II) complexes with this molecule and with two derivatives (D,L-piperidine-3-carboxylic acid and piperidine-4-carboxylic acid) were prepared, and the crystal structures of the complexes formed with the two derivatives were determined. The difference of the electron densities of carboxylate oxygen atoms derived from the position of the nitrogen atom resulted in interesting structural differences between the two complexes studied (**3a**) (**3b**).[48]

Although a number of studies have been published on the adsorption of amino acids on solid metal surfaces such as gold,[49,50] copper,[51,52] stainless steel,[53] platinum,[54] palladium[55] and silver,[56] little is known about the adsorption mechanism. Surface-enhanced Raman spectroscopy (SER) was used to study adsorption of a series of amino acids on electrochemically prepared silver. The results showed that the adsorption of all amino acids occurred *via* the ionized carboxylate group, and that the side chain of most of the molecules was also in close proximity of the surface. In contrast, the amino group was protonated and relatively far from the surface. Sulfur-containing amino acids also interacted through their sulfur atoms.[56]

Numerous ternary complexes formed with amino acids were prepared during the period reviewed.[57–70] In many cases, the studies stemmed from several key problems such as molecular/chiral recognition, modelling of biological systems and functions, and the relationship between molecular structure and *in vivo* behaviour of therapeutic agents. Non-covalent interactions, such as hydrogen bonding and hydrophobic and electrostatic interactions, are crucial factors for molecular recognition and contribute to the regulation in the formation of supramolecular structures. Ternary complexes involving aromatic diamines,



(3a)



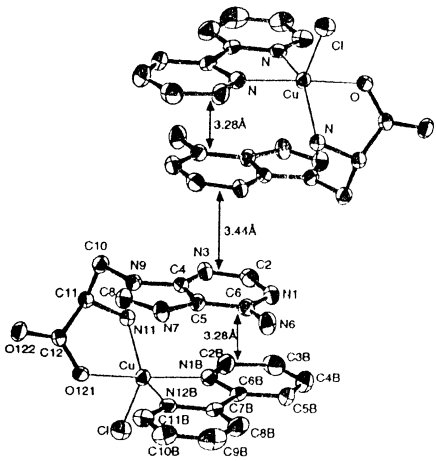
(3b)

2,2′-bipyridine (bpy) and 1,10′-phenanthroline (phen) are traditionally used to study non-covalent interactions.[57–60,65] Structural evidence for the existence of an aliphatic–aromatic CH–π interaction in Cu(II)-*p*-methylphenylalanine–phen and Pd(II)-*p*-methylphenylalanine–phen ternary complexes was provided by different spectroscopic and X-ray methods. However, no interaction between the coordinated *o*-methylphenylalanine and phen ligands was found.[59] In order to obtain more direct evidence for the specificity of the π–π interactions in nucleobases of DNA, two new amino acid derivatives, 1-(2-amino-2-carboxyethyl)adenine and 1-(2-amino-2-carboxyethyl)uracil, involving nucleobases adenine and uracil, were prepared. The ternary metal (M = Cu(II), Pd(II)) complexes of these two ligands with bpy or phen were also prepared and investigated. According to the results, the purine ring affords π–π stacking interaction both in solution and in solid states (**4a**), but the pyrimidine ring does not (**4b**).[65]

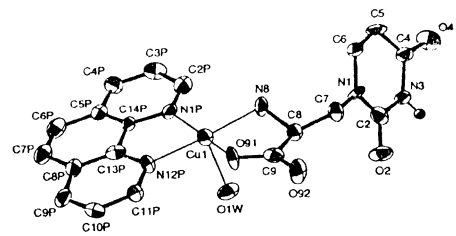
Fluorescent sensors based on molecular recognition are of particular interest. Two modified cyclodextrins 6-deoxy-6-*N*-[*N*α-(*N*^{2}-dansylaminoethyl)-*R*- (or *S*)-phenylalaninamide]-β-cyclodextrin, containing a metal binding site and a dansyl fluorophore, were prepared. Addition of D- or L-amino acids to their copper(II) complexes (**5**) induce a 'switch on' of the fluorescence which is enantioselective for Pro, Phe and Trp. The effect was used for the determination of the optical purity of Pro.[63]

There is much interest in designing chiral metal complexes that bind or transform their targets, whatever they may be, with high and predictable stereospecificity. Several chiral cobalt(III) complexes have been prepared for stereospecific recognition of natural amino acids.[66–69] Due to the relative position of the

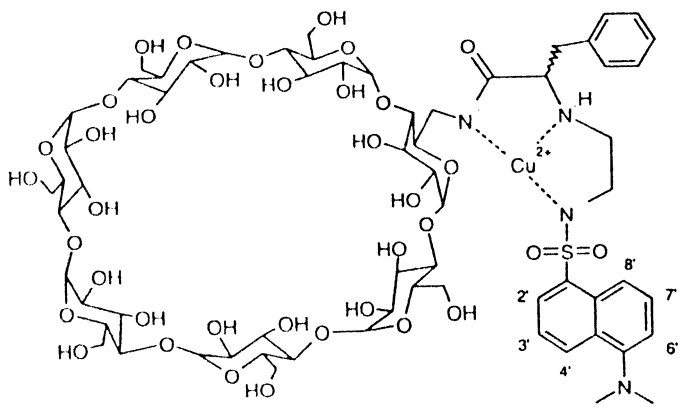
four coordinating atoms, the unnatural α-amino acid (**6a**), synthesized in a recent work, can form only one configurational isomer of its octahedral Co(III) complex. The two coordination possibilities of Ala to that complex are shown in (**6b**)



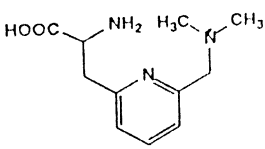
(4a)



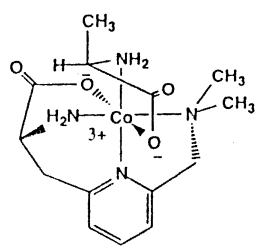
(4b)



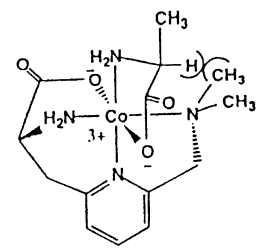
(5)



(6a)



(6b)



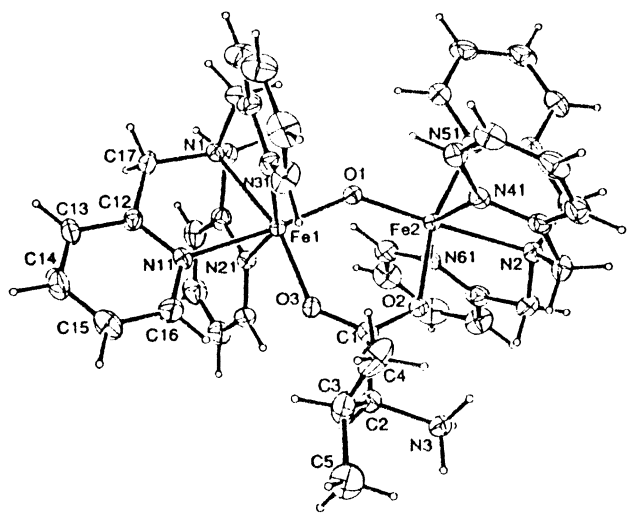
(6c)

and (**6c**). Both crystallographic analysis and NMR results indicated that the ratio of these two diastereomers in the crystal was about 60:40.[66]

Several additional new Co(tren)(amino acidato) ternary complexes, $[Co(tren)(D,L\text{-Phe})]\cdot(ClO_4)_2$, $[Co(tren)(L\text{-Phe})]I_2\cdot H_2O$, $[Co(tren)(L\text{-Pro})]I_2\cdot H_2O$, $[Co(tren)(L\text{-Trp})]\cdot(ClO_4)_2\cdot 1/2H_2O$, $[Co(tren)(L\text{-Val})]I_2\cdot 1/3H_2O$, $[Co(tren)(Ile)]I_2$ and $[Co(tren)(L\text{-Leu})]I_2$, were prepared and characterized by X-ray diffraction method. Their packing arrangements indicate that the complex cations in all of the compounds form helical strings, running along their crystallographic axes, for both chiral and racemic amino acidato species. In the racemic compounds, the complex cations of different strings have different chirality.[68]

The chemistry of oxorhenium(V) complexes is of great interest. It is widely accepted that the *in vivo* behaviour of the complexes depends very much on their structure. Following the '3 + 1' mixed ligand concept, the alternative '3 + 2' combination of the donor atoms (N,N,N/N,O) has been suggested. Five new cationic complexes containing the $[Re(V)O]^{3+}$ core, diethylenetriamine and an amino acid (Gly, Ala, Val, Leu or Pro), have been successfully prepared and characterized.[62]

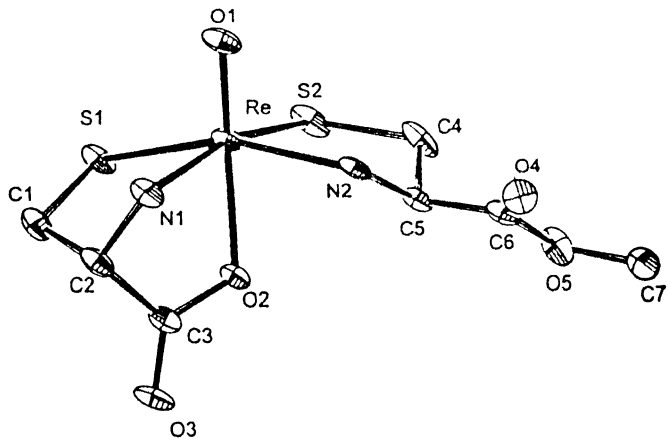
Numerous dinuclear metal complexes have been prepared as potential models for the active sites of different metalloenzymes. Synthesis and characterization of redox properties of a series of amino acid-bridged dinuclear iron(III) ternary complexes have been carried out. The general formula of the compounds prepared is $[Fe_2(\mu\text{-O})(\mu\text{-amino acid})(tpa)](ClO_4)_4$, where tpa = tris(2-pyridylmethyl)amine. As a representative example, the structure of the complex formed with L-Val is shown in (**7**).[70]



(7)

Modification of amino acids can significantly alter their metal binding ability or selectivity towards various metal ions. As a consequence, numerous different derivatives of amino acids have been prepared during the past two years and, in

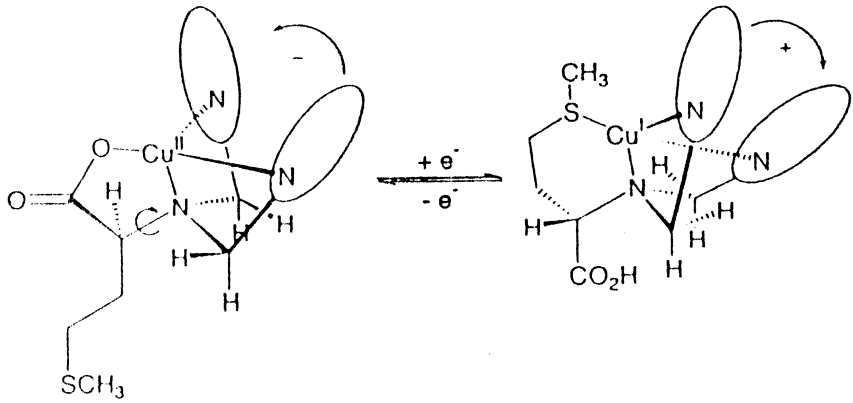
many cases, their metal complexation was studied. As models for Tc-labelled peptides, monooxorhenium(V) complexes of cysteine and cysteine methyl ester were synthesized and characterized. Unexpectedly, both ligands formed very similar 1:2 complexes with (S,N)(S,N,O) coordination. As is shown in (**8**), the Cys-OMe is partially saponified in its complex.[71]



(8)

Numerous previous results confirm that a substituent at the amino group reduces both the affinity of the amino N atom for metal ions with respect to the corresponding free α-amino acid and in-plane ligand field, which permits a relatively wide variety of coordination types, depending on the nature of the *N*-substituent. The role of *N*-alkyl chain length in water binding (or release) to the copper(II) coordination sphere in the [Cu(*N*,*N*-dialkyl-L-α-Ala)$_2$] complexes in various organic solvents has been investigated. It was found that while the water bound at the axial position to copper(II) was essential for the stability of the complex formed with the dimethyl derivative, apical water could easily exchange with water molecules from the solvent if the two ligands were diethyl derivatives, and the *N*-propyl chains screened the apical water from hydrogen bond-making solvents like methanol.[72] In order to investigate the influence of *N*,*N*-dialkyl chain lengthening on stereochemistry of the copper(II) alaninato complexes, a new force field (FF1) method has been developed.[73] Interesting redox-induced inversion of the chirality was observed in copper complexes of *N*,*N*-dialkylmethionines. In this study, the amino acid methionine was derivatized by the attachment of two chromophores to the nitrogen atom. The ligand obtained formed stable complexes with both Cu(II) and Cu(I), but with different coordination modes. While the three N donor atoms and the carboxylate were coordinated to the metal ion in the Cu(II) complex, the carboxylate and sulfide donors were exchanged in the Cu(I) complex. This change in the coordination mode caused inversion of the C– H twist and the chromophore orientational chirality (Scheme 1).[74]

N-Phthaloylamino acids were used to prepare new organotin complexes.



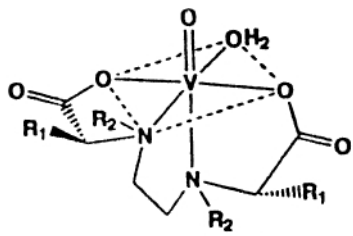
Scheme 1

Depending on the nature, number and arrangement of the organic groups attached to the tin atom, monomeric, tetrameric and polynuclear species were formed. Biological activity tests indicated powerful biocidal effects of the complexes prepared.[75] Dithiocarbamate derivatives of some naturally occurring amino acids were synthesized and their complexes with nickel(II) and copper(II) were studied.[76,77] Square-planar complexes were found with Ni(II), but Cu(II) was reduced to Cu(I).[76] Interestingly, both molybdenum(VI) and molybdenum(V) form complexes with a dithiocarbamate derivative of Pro. Reaction of the ligand with sodium molybdate leads to the formation of a biscomplex in which molybdenum can be found in its +6 oxidation state. The oxidation state of the metal centre, however, is +5 in the dimeric complex formed in the reaction of $MoCl_5$ with the dithiocarbamate in an inert atmosphere. The ligand is coordinated through its sulfur atoms in both types of complexes.[77] Complexes of the herbicide glyphosate, *N*-(phosphonomethyl)glycine, with various metal ions have already been studied. In a recent work, investigation of its complexes formed with a series of Group 2 (alkaline earth) metals has been carried out. It was found that although the general formula of the complexes formed with Ca(II), Sr(II) and Ba(II) is the same, $[M(HL)\cdot 2H_2O]$, only the structures of strontium glyphosate dihydrate and barium glyphosate dihydrate are the same, the structure of the calcium complex differs significantly, and all are different from the magnesium analogue, $[Mg(\mu_3\text{-}OH)_4(H_2O)_{12}]\cdot[Mg_2(L_2)(\mu\text{-}HL)(H_2O)_2]$.[78]

Several papers discuss results for oxovanadium(IV) complexes of *N*,*N*′-ethylenebis(amino acids).[79–81] Both the structures by X-ray and the insulin-mimetic activity of the complexes synthesized have been investigated. Schematic description of the bonding mode in the complexes is shown in (**9**) (n = the number of H_2O molecules in the complexes prepared).[80]

Both solution and solid state investigations on the metal complexes of several new N-protected amino acids, where the substituents are *N*-2-(4-amino-1-methyl-5-nitroso-6-oxo-1,6-dihydropyrimidinyl), *N*-2-(4-amino-1,6-dihydro-1-methyl-5-nitroso-6-oxopyrimidinyl), *N*-2-(6-amino-3,4-dihydro-3-methyl-5-nitroso-4-oxopyrimidinyl), *N*-(6-amino-3,4-dihydro-3-methyl-5-nitroso-4-oxo-

pyrimidin-2-yl) moieties, have been carried out. Since these ligands are able to coordinate to metal ions through the pyrimidine fragment and/or the amino acid moiety, they form metal complexes with different patterns ranging from mononuclear to 1D, 2D and 3D polynuclear.[82–85]

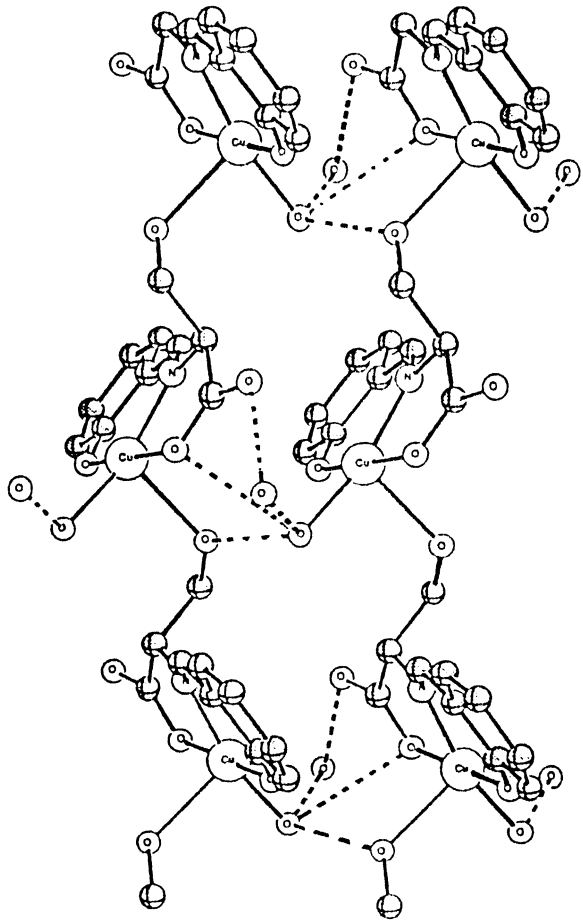


1 X = G (Gly), n = 0, $R_1 = R_2 = H$
2 X = (*S*)-A (Ala), n = 0, $R_1 = CH_3$, $R_2 = H$
3 X = (*S*)-V (Val), n = 2, $R_1 = CH(CH_3)_2$, $R_2 = H$
4 X = (*S*)-P (Pro), n = 0, $R_1 - R_2 = (CH_2)_3$
5 X = ^{m}G or G^{m} (Sar), n = 1, $R_1 = H$, $R_2 = CH_3$
6 X = (*S*)-M (Met), n = 0, $R_1 = (CH_2)_2SCH_3$, $R_2 = H$

(9)

Numerous complexes of amino acid-based Schiff bases with various metal ions such as Cu(II), Ni(II), Zn(II), Pb(II), Sn(IV), V(IV), lanthanide ions were prepared.[86–96] All kinds of the natural amino acids have been used to synthesize Schiff bases. The new compounds prepared show different biological effects and are all very effective metal chelators. Moreover, their metal complexes are potential models for a number of important biological systems. Copper(II) complexes of the Schiff base formed by the reaction of salicylaldehyde with L-Ser, $[Cu(Sal\text{-}(L\text{-}Ser))H_2O]\cdot H_2O$ and [Cu(Sal-(L-Ser))(2-amino pyridine)] have been prepared and characterized by X-ray diffraction. The interesting crystal structure of the binary complex consists of polymeric chains in which the coordination sphere of each Cu(II) is apically completed by a weakly bonded oxygen atom of the hydroxyl residue of an adjacent complex unit. The zig-zag chains interact by means of the carboxylate and the hydroxylic oxygen of one Ser moiety and two water molecules of the other unit (**10**).[86]

In order to study the relationship between structure and magnetic properties, a series of tridentate Schiff bases were prepared and their copper(II) complexes were characterized. The ligands were obtained by the condensation of amino acids β-Ala, D,L-3-aminobutyric acid, 4-aminobutyric acid and 2-aminobenzoic acid, with 2-imidazole- or 2-pyridinecarboxaldehyde. Direct influence of the amino acid side chains on the structure of copper(II) complexes was observed. Thus, where the amino acid chain contains two carbon atoms between the amino and carboxylic groups, helical-chain complexes are obtained; with three carbon atoms, the chain has sufficient flexibility to form a cyclic tetranuclear structure. All of these complexes exhibit weak ferromagnetic exchange interactions. Molecular structure of the tetranuclear copper(II) complex with the 4-aminobutyric

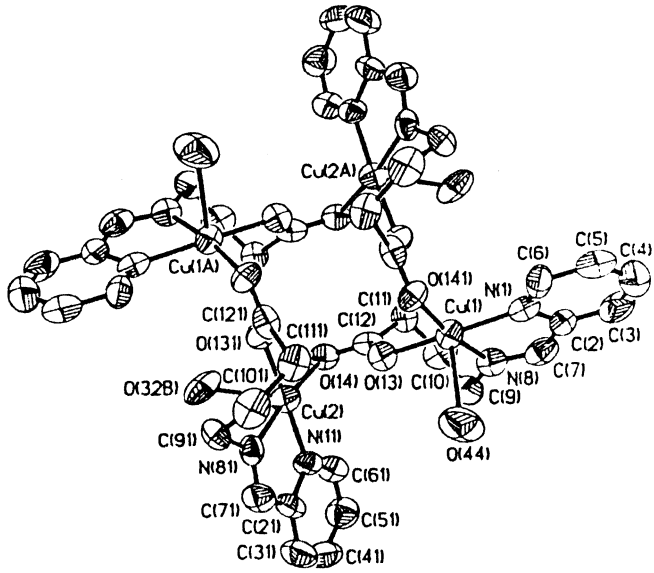


(10)

acid derivative is shown in (**11**).[87]

Several additional copper complexes with Schiff bases were found to show helical arrays in their crystals. The helical chain of the complex formed with *N*-[(5-methylimidazol-4-yl)methylene]-β-alanine is demonstrated in (**12**).[88]

Due to the discovered insulin-mimetic activity of some vanadium complexes, great effort is being made to find subsequent highly active, easily absorbable and low toxicity complexes. Well-chosen Schiff base complexes could be suitable candidates. A range of oxovanadium(IV) complexes of varying lipophilicity was prepared and characterized by using different methods. The complexes contain coordinated Schiff bases made from natural amino acids Gly, Ala, Val, Leu, Ile, Met, Phe, Thr, Asp and His, salicylaldehyde or derivatives, and one simple ligand like water, bpy or pyridine. In several cases, the solubility of the compounds from racemic amino acids was found to differ markedly from those containing the single enantiomer.[95]



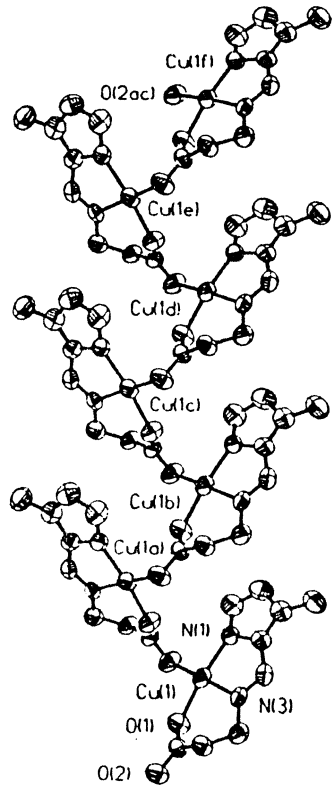
(11)

A large number of organometallic compounds containing amino acids/derivatives have been studied.[97–104] The reactions of the chloro-bridged complexes $[(p\text{-cymene})RuCl_2]_2$ and $[(C_5Me_5)IrCl_2]_2$ with 3-(3-pyridyl)-D-alaninate resulted in the formation of mononuclear half-sandwich complexes in which the amino acid is coordinated *via* its amino N atom and one of the carboxylate oxygens. Each complex contains one Cl^- anion coordinated to the metal ion. Abstraction of the chloride, however, allows the coordination of the pyridine N atom and dimers are formed. The structure of the Ir(III)-containing dimer is shown in (**13**).[97]

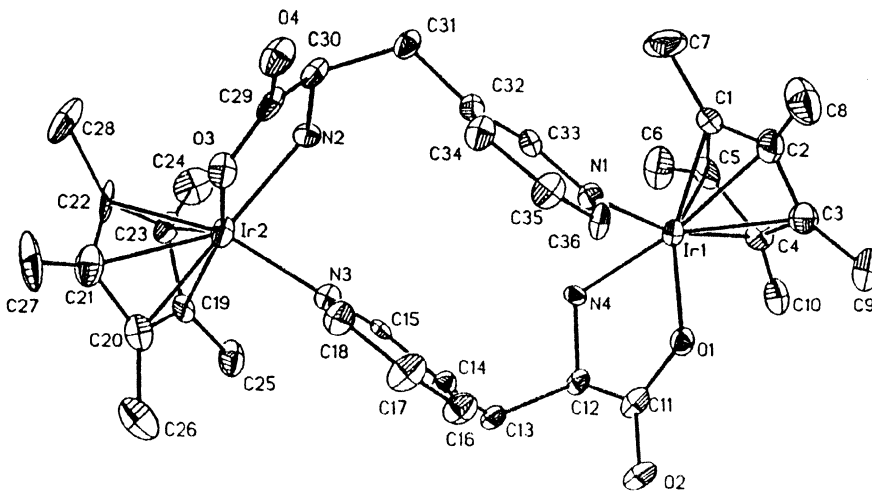
Ternary complexes are formed between amino acids and the newly synthesized chiral ortho-palladated palladium complex (*S*)-$[Pd\{C_6H_4(CH(CH_3)N(CH_3)_2)\text{-}C,N\}Cl]_2$ when this complex is used as a reagent for the determination of the enantiomeric ratio of the amino acids.[98] Organometallic labelling of amino acids and derivatives has recently gained considerable attention.[101–103] α-Amino acid esters were added to a carbonyl ligand of $[CpFe(CO)_3]CF_3SO_3$ to get the corresponding carbonyl complexes. The reaction is suggested for marking peptides at the amino end (**14**).[101]

In another paper, a general way of attaching amino acids to ferrocene derivatives by means of a Pd-catalysed two-step procedure for marking amino acids at the C-terminus is discussed.[102]

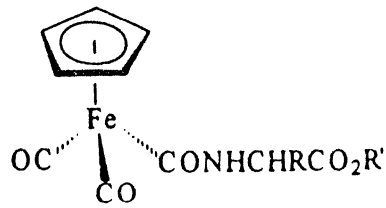
Because of their unique photophysical properties, polypyridyl ruthenium complexes find an increasing interest as luminescent structure/function sensors in the chemistry of biological macromolecules.[105,106] A series of $Ru(bpy)_3$-substituted amino acids (**15**) were prepared and investigated by steady-state and time resolved luminescence spectroscopy in a recent paper.


Cu(1f)
O(2ac)
Cu(1e)
Cu(1d)
Cu(1c)
Cu(1b)
Cu(1a)
N(1)
Cu(1)
N(3)
O(1)
O(2)

(12)

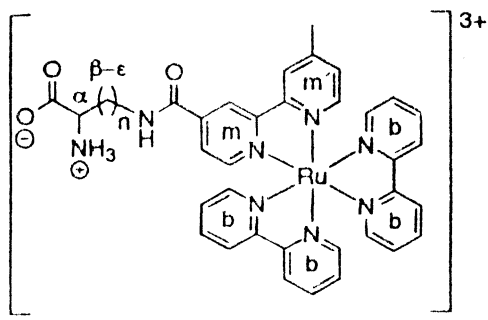

O4
C30
C31
C7
C29
C32
C1
C8
C28
O3
N2
C33
C6
C2
C24
C34
C5
N1
C22
C35
C3
Ir2
C23
C36
Ir1
C4
C27
C21
N3
N4
C10
C9
C19
C15
C20
C18
O1
C25
C14
C17
C12
C11
C26
C16
C13
O2

(13)



	R	R'
1	H	Et
2	Me	Me
3	$CHMe_2$	Me
4	CH_2Ph	Me

(14)



n = 1 : $[H_3N\text{-}DAPA(Rub_2m)\text{-}OH]^{3+}$ (1)

n = 2 : $[H_3N\text{-}DABA(Rub_2m)\text{-}OH]^{3+}$ (2)

n = 3 : $[H_3N\text{-}Orn(Rub_2m)\text{-}OH]^{3+}$ (3)

n = 4 : $[H_3N\text{-}Lys(Rub_2m)\text{-}OH]^{3+}$ (4)

(15)

Due to the absence of significant electronic interactions between the ruthenium chromophore and the amino acid moieties, the absorption spectra of the studied complexes did not change as a function of pH. The luminescence intensities of the complexes, however, showed a marked dependence on pH. The results demonstrate how the protonation (or deprotonation) of a remote amino acid group can significantly modify the photophysical properties of a chromophore linked to a biologically relevant molecule.[106]

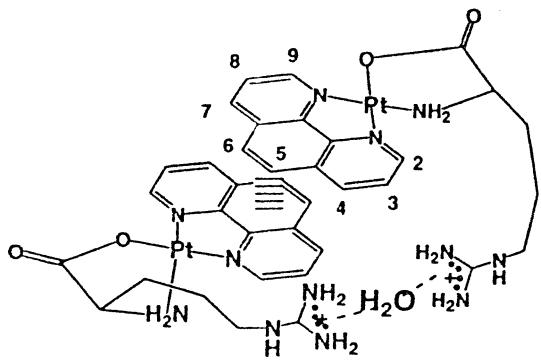
Sodium and potassium and, to a lesser extent, lithium are known to have important effects in biological systems. To gain a better understanding of the interactions of these metals with large biological molecules, several studies on model systems have been carried out.[107–116] For example, the complexes of

D,L-homocysteic acid with Na^+, K^+ and Ca^{2+} were synthesized and studied by FTIR and FT-Raman spectroscopic methods. The results show that the carboxyl and sulfonate groups are both coordinated to these metal ions.[107] The suggestion that the aromatic side chains of Phe, Trp and Tyr should be strong donors for Na^+ and K^+ in biological systems has initiated studies on such interactions.[108,109] With the help of a synthetic receptor molecule incorporating phenolic side chain of Tyr, the first evidence for K^+–π interaction has been observed recently.[108] In numerous cases theoretical calculations have been performed to establish alkaline affinities of various amino acids such as Gly,[110,111,115,116] Ala,[111,115] Ser,[110] Pro,[110] Arg,[112,113] Phe,[114] Trp,[114] Tyr,[114] in the gas phase. In several cases, theoretical calculations are combined with investigations performed in the gas phase. The importance of mass spectrometry is increasing rapidly in this field, largely due to the emergence of electrospray ionization (ESI) and matrix-assisted laser-desorption ionization (MALDI). Mass spectrometry is a unique method because of its intrinsic sensitivity and molecular specificity, and because the interactions are studied in the absence (or using small controlled amounts) of solvent. *Ab initio* and molecular mechanics calculations have also been made for some other systems such as zinc(II)-Gly,[117] copper(I)-Val, -Lys, -Arg,[118] aluminium(III)-Asn and -Gln,[119] magnesium(II)-Asn and Gln,[119] and for the interaction of Gly with alkaline earth metal ions[120] and a series of amino acids with copper(II).[121] Ligand field calculations for several Cr(III) complexes of Gly, Ser and Leu have been performed.[122]

2.2 Solution Equilibria. – In addition to pH-potentiometry, different spectroscopic methods, *e.g.* UV-visible, IR, NMR, EPR, electrochemical methods (including a quite new technique, capillary electrophoresis) and calorimetry were used to determine the stoichiometry, stability, thermodynamic data and bonding mode of species formed in solution. In many papers both solution and solid state results are discussed. Owing to the permanent interest in these kinds of studies for several decades, an enormous amount of data was previously determined especially for simple binary systems. In spite of that fact, numerous data for metal ion–amino acid binary systems appeared during the period of this coverage. Equilibrium results for metal complexes of interesting derivatives of amino acids, and for ternary systems have been published, and molecular recognition problem has attracted special interest in recent years.

Within the subject of complex formation between metal ions and simple natural amino acids, different motives have initiated reinvestigations of some previously studied systems.[123–128] For example, to study the effect of surfactant micelle on the stability constants, Cu(II)-Gly and Cd(II)-Gly systems in the presence of sodium dodecyl sulfate and cetylpyridinium nitrate ionic surfactants were investigated.[123] Solution equilibrium studies on copper(II)-Orn and copper(II)-Lys systems have already been carried out. In a recent work, results of combined potentiometric, calorimetric, UV-visible and ESR measurements have provided further support for the coordination of the side chain amino group in the copper(II)-Orn complexes only.[125] Non-covalent interactions play an important role in many biological processes such as molecular recognition, enzymatic

reactions and protein structure stabilization. The guanidinium group in the side chain of Arg may have vital functions in specific non-covalent interactions. In order to study this problem in more detail, spectroscopic and potentiometric measurements for the copper(II)-L-Arg and copper(II)-L-Lys systems using 0.1 M guanidinium chloride as a background salt were performed. Well-defined adducts between the copper(II)-L-Arg complexes and guanidinium cations were found. The complexes containing L-Lys, however, did not show such an interaction. In this work ternary complexes were also investigated and NMR results for the platinum(II)-phen-L-Arg system suggested the formation of different adducts with different self-association constants (log K_D). One type of adduct, shown in (**16**), involves Arg guanidinium . . . H_2O . . . Arg guanidinium interaction.[127]



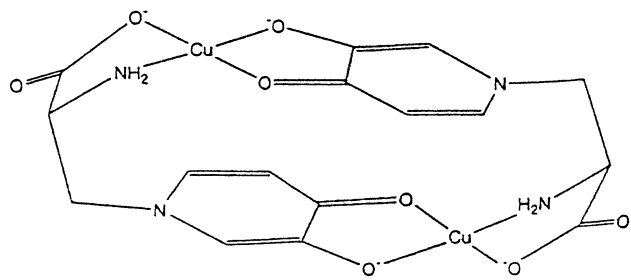
log K_D = 1.23

(16)

Some new thermodynamic data for the Zn(II)-Hys 1:1 complexes formed in presence of counter anions SO_4^{2-}, NO^{3-}, OAc^- and Cl^-, were determined.[128] In spite of the great interest in vanadium complexes, very few equilibrium studies for vanadium–amino acid systems have been performed so far. Investigations on V(V)-Asn[129] as well as on V(IV)-Asp and V(IV)-Glu systems[130] produced some new data in this field. Simple amino acids are not the best ligands for typical hard metal ions such as lanthanides. Moreover, the great affinity of these hard metal ions toward hydroxide ion makes the equilibrium systems quite complicated. Eight amino acids and lanthanum have been involved in a recent calorimetric work in which the stability constants and thermodynamic functions have been determined. The enthalpy change was found to be a predominant contribution to the stability of the complexes. The higher stability of the 1:1 complexes formed with ring-containing amino acids was assigned to the interaction between the ring and lanthanum. On the other hand, steric effects between the rings led to the significantly less favoured coordination of 'second' ligands.[131] Eu(III)-Gln and Eu(III)-Ser solution equilibrium systems have been investigated in another study.[132] Traditionally, the very inert platinum(II) is modelled by palladium(II) in studies, still there are relatively few publications describing solution equilibria of palladium(II)-amino acid systems. Potentiometric and spectrophotometric measurements were used to calculate the stability constants and determine the

bonding modes of the palladium(II) complexes with sulfur-containing amino acids and derivatives, *e.g.* L-cysteine, D,L-penicillamine, *S*-methyl-L-cysteine, D,L-methionine, D,L-ethionine. As had been expected, thiolate and thioether sulfur atoms play an essential role in the complex formation in the systems studied. A comparison between the stability constants of the Pd(II) complexes and the corresponding Hg(II) ones revealed the higher stability of the Pd(II) complexes.[133] ^{1}H, ^{13}C and ^{195}Pt NMR studies provided results for transformations of Pd(II) and Pt(II) complexes with Gly and Ala in DMSO and H_2O solvents.[134,135]

L-Mimosine, α-amino-β-(3-hydroxy-4-oxo-1,4-dihydropyridin-1-yl)propanoic acid is a relatively rare plant α-amino acid. This molecule contains two different chelating residues, therefore, alternative coordination modes are available for metal binding. While the maltol-like coordination mode was found with V(IV) and Zn(II), the mixed-type bonding mode was preferred in the Ni(II) complexes. The very stable dinuclear complex involving both maltol-type and amino acid-type chelates is predominantly formed in the copper(II)-L-mimosine system (**17**).[136]

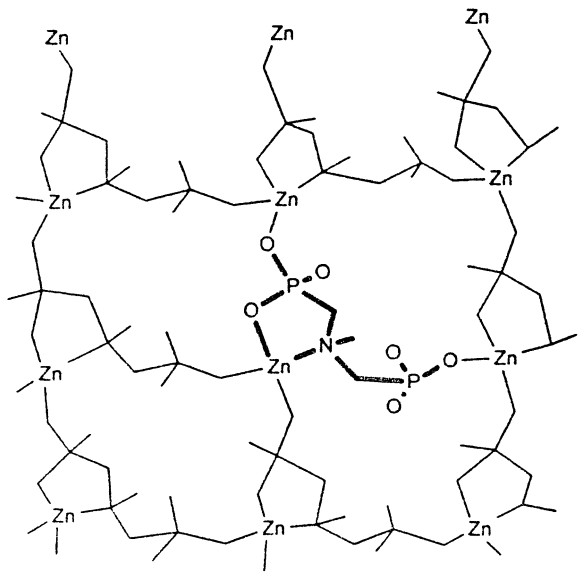


(17)

Most equilibrium studies have been performed in aqueous solution and only a few stability constants have been determined in mixed solvents.[137–139] A spectrophotometric method was used to study the complexation between Leu and dioxovanadium(V) in methanol–water and dioxane–water mixtures and linear relationships were observed when logarithmic stability constants were plotted *vs.* $1/D$ values (D = dielectric constant).[137]

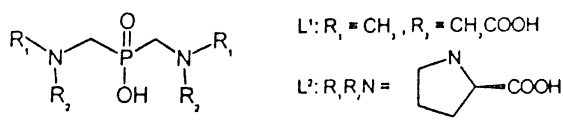
Metal complexation of numerous interesting derivatives and analogues of amino acids were investigated during the period reviewed. Close correlation is often found between the protonation constants of ligands with similar structures and the formation constants of their complexes. This type of correlation relating to Cu(II) complexes of Gly and a series of its *N*-alkylated derivatives was evaluated in a recent publication.[140] Aminoalkylphosphonic acids are broadly defined as amino acid analogues in which one or more carboxylic groups are replaced by phosphonic or related functions. Owing to the biological importance of such studies, many solution equilibrium investigations on the metal complexation of different aminophosphonic and aminophosphinic acids have been made during the last few decades. Most of the results published before 2000 have been summarized in a recent review.[8] In more recent papers Mn^{2+}, Co^{2+} and Ni^{2+}, as well as Ca^{2+}, Mg^{2+} and Zn^{2+} complexes formed with a series of aminodi-

phosphonates, have been studied by potentiometric and different spectroscopic methods.[141,142] Pure phosphonate coordination was found in most cases, but tridentate bonding *via* two joined five-membered chelates predominated with zinc(II), except in more concentrated solutions of the zinc(II)-*N*-methyl-iminodimethylene-diphosphonic acid and the zinc(II)-iminodimethylene-diphosphonic acid systems, where interesting polymeric species were formed (**18**).[141] According to other results, the involvement of pyridine, pyrazole or imidazole moieties in the side chains of aminomethylphosphonates increases considerably the copper(II) binding power of the ligands. The most stable complexes were found with the imidazole-containing derivative.[143]



(18)

Two sarcosine and L-proline 'units' are linked together with a bis(methylene)-phosphinate group in two new ligands (**19**). The complexation properties of these ligands towards Mg(II), Ca(II), Cu(II), Zn(II), Cd(II) and some lanthanides have been investigated. The in-chain phosphinate group was found to participate in complex formation with all metal ions except Cu(II). Most probably, it is the tetragonal structure of the Cu(II) complexes that is responsible for this difference.[144]



(19)

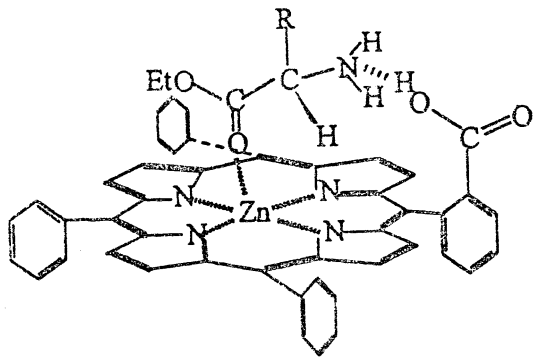
Hydroxamic acid derivatives of amino acids have many effects on biological systems and at the same time they are very effective chelating agents. That may be the main reason why there has been special interest in metal complexation

studies of aminohydroxamic acids for decades. These ligands are known to coordinate 'soft' metal ions *via* their amino-N and hydroxamate-N atoms and 'hard' metal ions *via* their hydroxamate oxygens. In molybdenum(VI)-α-aminohydroxamate complexes interesting changes in the coordination mode with changing pH were found. While two ligands through their hydroxamate oxygens are coordinated to a MoO_2^{2+} core in the acidic pH-range, one ligand *via* its amino-N and hydroxamate-N atoms is coordinated to a MoO_3 core at neutral pH and in slightly basic solutions.[145] On the other hand only the hydroxamate oxygens are coordinated to vanadium(V) in the pH-range 2.5–10.5.[146] 2-(Hydroxyimino)propanohydroxamic acid (an analogue of alanine) contains five potential donor atoms, three oxygens (one oximic and two hydroxamic) and two nitrogens (one oximic and one hydroxamic). This rather unusual donor arrangement makes this small molecule a very effective ligand for many metal ions, including aluminium(III),[147] nickel(II) and copper(II).[148]

Numerous solution equilibrium studies for ternary systems have been made over the last two years.[149–185] If one looks at the subject of the corresponding papers, it seems as if bpy, tren and other simple amine 'secondary' (B) ligands have been used in somewhat fewer investigations than before, but they are still involved in many studies.[149,150,163,166] On the other hand, 'more bio' B ligands such as vitamins B_1,[167] B_5,[174] B_6,[156,177] folic acid,[173] ATP,[153] inosine, cytosine[171] have been increasingly chosen. Compared to the numerous stability constants published during the period of coverage, there are far fewer examples of thermodynamic data.[152,167,174] The factors affecting the stability of ternary metal complexes, such as electrostatic, hydrophobic or steric interection between the ligands, have been extensively studied and, in many cases, the bonding modes have also been discussed. Due to the hydrophobic interaction or the aromatic ring stacking, stability-enhancement is often displayed by ternary complexes involving aromatic B ligand such as bpy, phen or their derivatives.[149,157,166,167,176] 2,2′-Dipicolylamine, similar to bpy, has two heteroaromatic pyridine donors. Based on the result that its copper(II) complexes containing aromatic amino acids are significanly more stable than the complexes containing alkyl or hydroxyalkyl-substituted amino acids, the presence of intramolecular interaction between the hydrophobic moiety of 2,2′-dipicolylamine and the aromatic side chains in the solution was suggested. In solid state, however, no intramolecular ring stacking between the pyridine ring of 2,2′-dipicolylamine and the phenyl ring of /-Phe was found. Instead of this, intermolecular stacking was observed between the pyridine rings of adjacent complex molecules.[151] Cyclic voltametric results indicated that the strong charge-transfer interaction between the sulfur atom of Cys as electron donor, and the pteridine ring of folic acid as electron acceptor, is the main component of the forces holding together an interesting adduct in the $Cd(Cys)_2$-folic acid system. The observed interactions may serve as a simplified model of processes occurring between folates, proteins and metal ions in living systems.[173]

Molecular/chiral recognition problems are always the focus of interest. Continuing efforts to develop an artifical water-soluble receptor comparable to proteins has resulted in a series of zinc(II) complexes of porphyrin based compounds

with a hydrophobic binding pocket. These receptor molecules were found to bind various amines, α-amino acid esters and oligopeptides with high selectivity.[179] Unsymmetric tetraphenylporphyrin derivative bearing a carboxyl group at one of the four phenyls in the porphyrin was constructed in another study. An interesting type of binding of amino acid ethyl esters to the zinc(II) complex of this new porphyrin derivative (**20**) was found.[180]

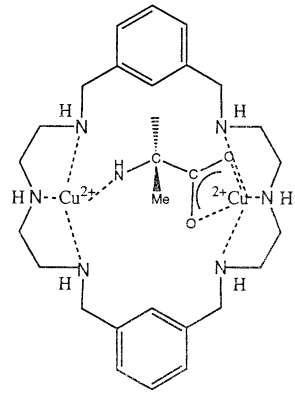


(20)

Enantioselective complexation of amino acids by chiral selector complexes has been investigated in some laboratories.[159,181,182] Chiral recognition of α-amino acid esters on the homochiral helical dimer of the zinc(II) complex of linear tetrapyrrole (zinc bilinone) has been achieved recently.[181] Studies of complex formation between metal(II) complexes of 6(A)-deoxy-6(A)-hydroxyethylamino-β-cyclodextrin and various amino acids have been carried out. The most stable ternary complexes were formed if copper(II) complex was used as a selector, but the greatest enantioselectivity was found with the nickel(II) complex, and none with the zinc(II)-cyclodextrin complex. These results led to the conclusion that there is no direct correlation between the stability of the complexes and the selectivity achieved.[159] In another study it was found that the enantioselectivity of binding amino acids to chiral copper(II) complexes was highly affected by the size and the character of side chains.[182] Macrocyclic polyamines are versatile chelating agents. The dinuclear copper(II) complex of the macrocyclic ligand 3,6,9,17,20,23-hexaazatricyclo[23.3.1.1^{11,15}]triaconta-1(29)11(30),12,14,25,27-hexaene was used as a host molecule for various amino acids (**21**).[161,162] If Ala, Val, Leu, Nle, Nva and Ser were used as guest ligands, due to the large inductive effect and the linear structure of the side chain of Nle, this latter ligand formed the most stable complexes.[161] Additional donor groups in the amino acids such as β-carboxylate of Asp and imidazole of His have a strong effect on the interaction.[162]

Some new results for solute–solvent interactions were obtained when solubility measurement technique was used to study the interaction between different salts and different isomers of tris(L-amino acidato)cobalt(III) complexes in solution.[186,187]

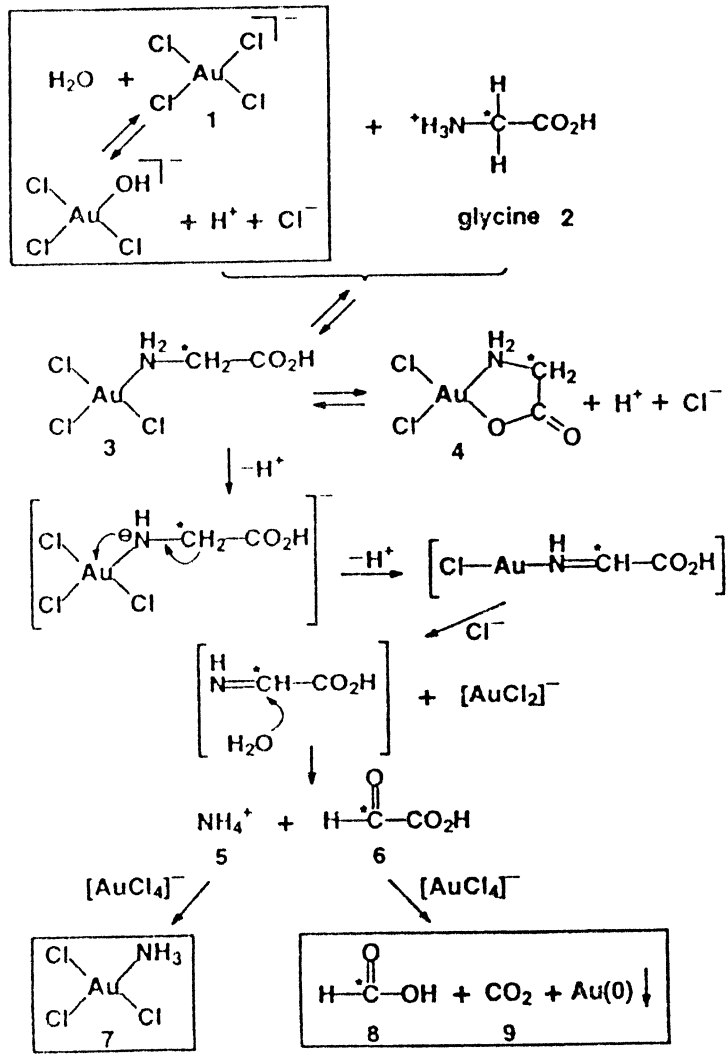
2.3 Kinetic Studies. – Most of the kinetic works on metal ion–amino



(21)

acid/derivative complexes deal with oxidation of amino acids by high oxidation state metal ions.[188–209] Frequently used oxidizing agents are permanganate (both under acidic and basic conditions),[190–192,195,196,203,206,208] vanadium(V),[189,193,194,198,204] osmium(VIII),[197] chromium(VI),[201] diperiodatonickelate(IV)[202,205] and diperiodato-argentate(III).[188] Diperiodatoargentate(III) was used to oxidize Gly but the actual reactive species was $[Ag^{III}(H_2IO_6)(H_2O)_2]$. Initially the reactive species binds one Gly, probably through the amino N atom, in a fast step to produce Ag(III)-monoglycinato complex. Then this intermediate takes up another ligand. The biscomplex is decomposed by transference of an electron from the ligand to the silver(III) centre. The silver(II) intermediate thus formed oxidizes another ligand attached to the complex yielding eventually the products of decarboxylation and deamination.[188] In view of the toxic effects and antitumour activity of some gold(III) compounds it is important to understand the chemistry of Au(III)-biomolecule interactions. Surprisingly, the first results for Au(III)-induced oxidation of Gly were published only recently.[207] NMR investigations of otopically-labelled Gly showed deamination of the amino acid with formation of glyoxylic acid, NH_4^+, formic acid, CO_2 and metallic gold. The proposed mechanism is shown in Scheme 2.

Several papers discuss oxidation of Cys/derivatives by alkaline permanganate,[208] potassium ferrate[209] or dioxygen.[210–212] Kinetic data for the oxidation of L-Cys by alkaline permanganate suggest that the oxidation proceeds *via* the formation of a complex between L-Cys and permanganate which decomposes slowly, followed by a fast reaction between the free radical of L-Cys and another molecule of permanganate to give the products.[208] Studies on copper-catalysed oxidation of Cys resulted in the interesting conclusion that catecholamines, including epinephrine, norepinephrine, dopamine and dihydroxyphenylalanine accelerate the oxidation of Cys under aerobic conditions, while they have no effect under anaerobic conditions. On the other hand, phenolic amines such as tyramine and tyrosine neither accelerate nor inhibit the autoxidation.[211] OsO_4 catalysed oxidation of Met by an organic agent chloramine-T in alkaline medium has also been investigated.[213] Similarly, some new results for metal ion

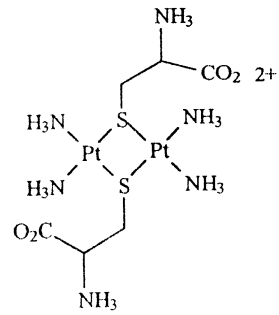


Scheme 2

catalysed reduction of amino acids have been published.[214,215] Since γ-hydroxylated α-amino acids are common building blocks of bioactive compounds, a number of methods for stereoselective synthesis of such compounds have already been developed. In a recent paper, a manganese(II) chloride-catalysed, highly stereoselective direct reduction method was published for the reduction of γ-aryl-γ-oxo-α-amino acids to γ-hydroxylated α-amino acids.[214]

Platinum(IV) complexes undergo ligand substitution reactions much more slowly than their platinum(II) analogues, and are therefore usually seen as prodrugs for platinum(II) compounds. For this reason, the platinum(IV) species can be activated by reduction to their platinum(II) analogues. Since thiol- and

thioether-containing biomolecules are amongst the major cellular reductants, sulfur-containing amino acids and derivatives were chosen to investigate the kinetics of reduction of some platinum(IV) prodrugs. It has been found that the reduction is strongly pH dependent, being related to the protonation state of the amino acid and the basicity of the sulfur.[216,217] Reactions of some hydrolytic products of cisplatin with Met and Cys showed significant differences. While Met is unreactive toward *cis*-[PtCl(OH)$(NH_3)_2$] at pH = 7, Cys reacts readily and a sulfur-bridged dimer (**22**) is predominantly formed.[218] Similar differences between the reactivity of Cys and Met toward [Pd(Cl)(terpy)]$^+$ were observed. *S*-Methyl-L-Cys, like Met, was also unreactive toward [Pd(Cl)(terpy)]$^+$ at pH = 7. All these results confirm that the methyl substituent of the sulfur atom is responsible for the differences, because it hinders the formation of the sulfur-bridged dimeric species.[219]



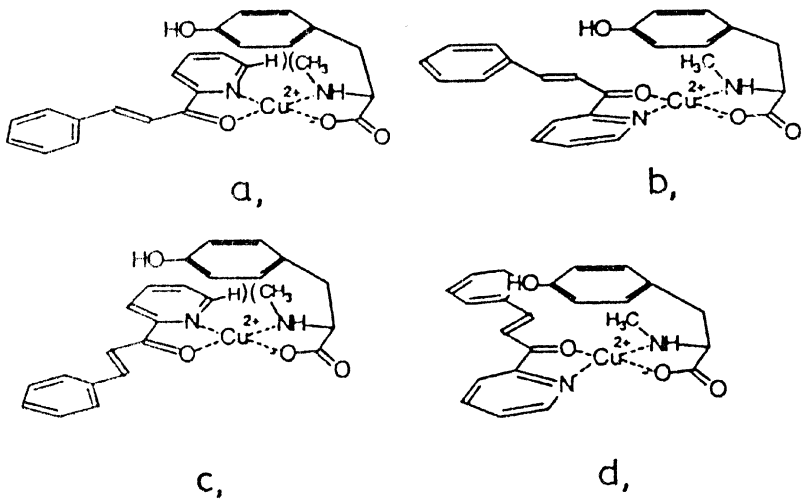
(22)

Some new results for substitution reactions involving amino acid complexes appeared in the period of coverage.[220,221] The substitution reactions on *cis*-[Pd(L-His)(H_2O)]$^{2+}$ by chloride and nucleobases and dinucleotides have been studied. Since irreversible polymerization reactions hindered the studies at neutral pH, the measurements were made in acidic solutions (in the pH range 1–3).[220] The kinetics of the reaction of [Cu(biguanide$)_2$]$^{2+}$ with an excess of different amino acids like β-Ala, L-Phe and L-Val have been investigated in aqueous solution. In all these systems, the ligand replacement process was suggested to pass through an intermediate formation of the ternary complex as the rate determining step, followed by the rapid transformation into the [Cu(amino acidato$)_2$] complex. The reactivities were compared with those of some other ligands and, as a result, the following reactivity order was found: Gly > α-Ala > β-Ala > Val > Leu.[221] Additional kinetic studies resulted in some new data for complexation of chromium(III) with D,L-Leu,[222] nickel(II) and cobalt(II) with L-2,6-diaminocaproic acid,[223] for metal ion catalysed oligomerization of L-γ-carboxyglutamic acid.[224] Rate constants were determined by means of optical rotation measurement for isomerization processes taking place in partially resolved [Ni(phen$)_2$(*S*-amino acid)] systems.[225]

2.4 Synthetic, Analytical and Biomedical Applications of Amino Acid Complexes. – A huge number of papers published during the past two years can be

associated with these subjects. However, many of them are beyond the scope of this chapter or are summarized in other parts of this volume, *e.g.* in those dealing with different metal ion catalysed routes of synthesis to various amino acids. Consequently, here only several papers being closely related both to metal complexation and application of amino acids/derivatives are mentioned.

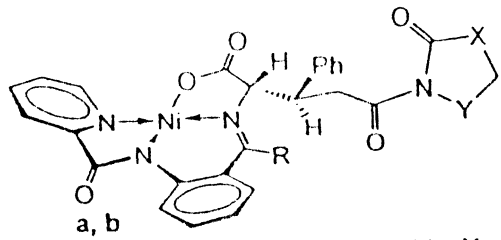
The influence of amino acids coordinated to metal ion catalysts on the rate and particularly the enantioselectivity of organic reactions is extensively studied nowadays. The effect of a series of diamines and α-amino acids on the rate and enantioselectivity of the Ni(II)- and Cu(II)-catalysed Diels–Alder reaction between 3-phenyl-1-(2-pyridyl)-2-propen-1-ones and cyclopentadiene in water has been investigated in a recent paper. Interesting ligand accelerated catalysis has been observed for several aromatic α-amino acid ligands, which can be assigned to arene–arene interaction between the aromatic ring of the α-amino acid and the pyridine ring of the dienophile in the ternary complexes formed in the systems. Four different geometries can be adopted in the ternary complexes. These geometries differ with respect to the coordination environment around the central copper ion (*cis* or *trans*) and the conformation of the α,β-unsaturated ketone (*cisoid* or *transoid*) (**23a**,**b**,**c**,**d**). Due to a steric repulsion which arises between the pyridine α-hydrogen atom of the dienophile and the methyl group of the α-amino acid in the *cis* complexes (**23a** and **23c**) and which is absent in the *trans* complexes (**23b** and **23d**), *N*-methylation of amino acids significantly influenced the enantioselectivity.[226]



(23)

Nickel(II) complexes of Gly based Schiff bases were used to develop a practical method (proceeds at room temperature) for stereo-controlled synthesis of 3-substituted pyroglutamic acids. The diastereomerically pure intermediate (**24**) can be easily decomposed to the final products.[227]

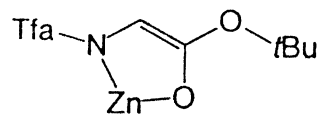
Enantioselective reduction of prochiral ketones by chromium(II)–amino acid complexes were performed in other studies.[228,229] Zinc(II) complexes formed with



a, b

R = Ph; X = O; Y = CH_2 (a); R = Me; X = O; Y = CH_2 (b);

(24)

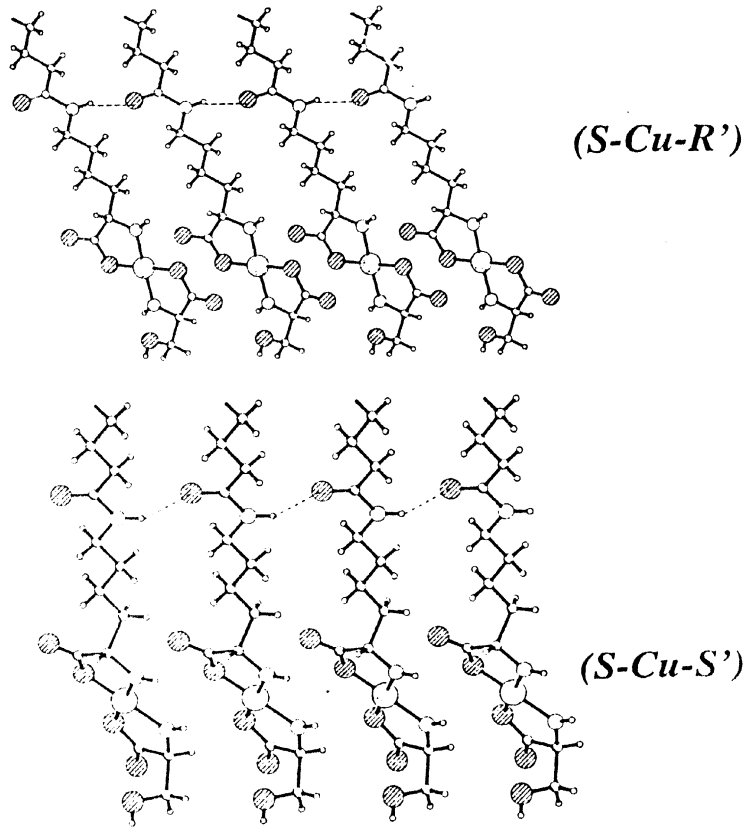


(25)

enolates of different N-protected amino acid esters were used as nucleophiles in palladium-catalysed allylic substitutions. Tfa (= trifluoroacetyl moiety) is the protecting group of the ligand in the complex shown in (**25**).[230] Development of chiral building blocks for transition metal catalysts and biomimetic assemblies was the main goal when chiral quadridentate derivatives of L-Phe and L-Lys were synthesized and their zinc(II) complexes were prepared.[231]

To monitor structural organization of chiral molecules during the early stages of crystal nucleation is of importance in pure and applied sciences. Clusters formed in supersaturated solutions play an ubiquitous role in crystallization processes. One way to obtain information on interactions between clusters and chiral molecules is by studies on the oriented growth of crystals at an air–solution interface. Interaction between clusters of polar headgroups of monolayers of the copper complexes of S-Cu-S′ and S-Cu-R′ and water soluble copper complexes S′-Cu-S′ and R′-Cu-R′ were investigated (where S represents enatiomerically pure (*S*)-palmitoyl-N^{ε}-Lys and S′ and R′ represent chiral resolved (*S*) and (*R*) forms of Ala, Ser or Val). According to the results, the S-Cu-S′ and S-Cu-R′ monolayers have different structures (**26**) and the S′-Cu-S′ biscomplexes can enantioselectively bind to the periphery of the domains of the *cis*-S-Cu-S′ monolayers, but not to the *trans*-S-Cu-R′.[232]

Due to the explosive growth in interest in the synthesis and application of enantiomerically pure compounds, analytical methods for the separation and determination of enantiomers have also become very important in the past few years. Some new methods are now available for this determination by means of various chiral metal complexes, using first of all NMR, CD and MS methods.[233–239] Isomeric amino acids Leu and Ile can be readily distinguished and quantified in 90:10 to 10:90 mixtures by MS2 and MS3 tandem mass spectrometric dissociation of their ternary complexes formed with copper(II) and bpy.[240] Relatively stable five-coordinated trigonal-bipyramidal [$Pd(pp_3)X$]-type complexes (where pp_3 = tris(2-(diphenylphosphino)ethyl)phosphine, X = Cl^-, Br^-,



(26)

I^-) can selectively discriminate the thiolato sulfur atom from the other donor atoms of amino acids. This discrimination is based on the sulfur coordination in the axial position and it is suggested that the method be used for the separation and determination of L-Cys or glutathione. The oxidized form of glutathione which has a disulfide bond instead of the thiolate group, does not react to form a thiolato complex.[241] In many cases, ternary complex formation and ligand exchange processes constitute the chemical basis of the new methods developed for chiral separation of various amino acids.[242–245]

The biological importance of metal complexes of amino acids has initiated numerous studies. Some of the previous results are discussed in a special issue of *Chemical Reviews*,[246] others are cited in the former parts of this chapter. Some additional papers presenting different biological models[247–251] or discussing *in vivo* effects of metal complexes of amino acids/derivatives are mentioned here.[252–263] Far-advanced self-organized structures exist in many biological systems. Self-organization and structure-determining factors in the $[Cu(\text{L- or D-Arg})_2]^{2+}$-containing systems in the presence of dinegative tetrahedral anions such as sulfate, 1,3-benzenedisulfonate (*m*-bs^{2-}), isophthalate (*m*-pa^{2-}), pyridine-

2,6-dicarboxylate (2,6-dp^{2-}), pyridine-3,5-dicarboxylate (3,5-dp^{2-}), were clarified in the solid state by X-ray and in solution by various spectroscopic methods. Depending on the nature of the anion, self-organized right- and left-handed double-helical, right-handed single-helical, and layer structures were formed (Scheme 3).[247]

The stacking of parallel base pairs in DNA was modeled by a ternary complex of Phe, Δ-α-[Co(*N*,*N*′-dimethyl-*N*,*N*′-di(2-picolyl)-1*R*,2*R*-diaminocyclo-hexane)(*S*-Phe)]$(ClO_4)_2 \cdot H_2O$. The strong intramolecular π–π interaction existing in the complex is shown in (**27**).[247]

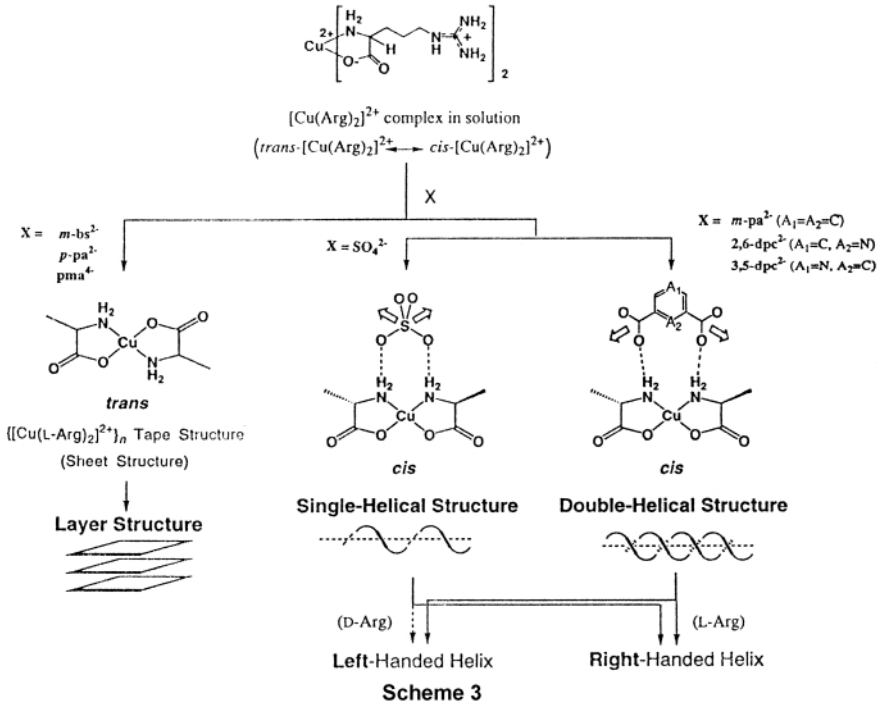
In a few cases metal complexes have been studied as models for active centres of various metalloenzymes or other biomolecules.[249–251] Antitumour, antibacterial effects of several metal complexes of amino acids/derivatives have recently been discovered. Besides some new platinum(II) and palladium(II) complexes,[255,256,261,262] some organotin,[257] lanthanide,[258] gold,[259] and copper[260,262] complexes were also found to be biologically active. Simple amino acids as well as derivatives, like the thiourea derivative of L-Leu methyl ester[262] (**28**) were involved in these studies.

Several newly synthesized oxovanadium(IV) complexes of N-substituted amino acids are suggested as antidiabetic agents and antihypertensives.[263]

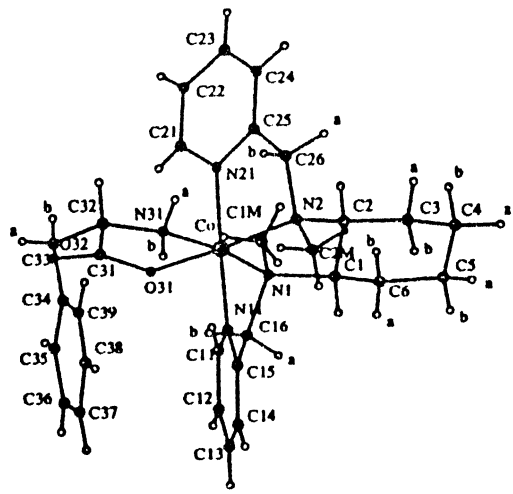
3 Peptide Complexes

3.1 Synthesis and Structural Studies on Peptide Complexes. – A great number of new peptide complexes containing a wide variety of metal ions have been synthesized and structurally characterized in the past two years. These studies were mainly devoted to the complexes of metal ions with outstanding biological significance and the roles of various strongly coordinating side chain residues of peptides. Widespread clinical applications of platinum complexes in cancer chemotherapy resulted in many publications on the platinum(II/IV) and palladium(II) complexes of peptide ligands.[264–268] It is clear from these studies that simple dipeptides are very efficient and versatile ligands for the complexation with divalent metal ions. The structures of the various species depend on several internal and external parameters, including the presence and location of the side chain residues and pH of the solutions. Bidentate chelation of the N-terminal amino acid residue was described in the [Pt(L-Met-Gly)Cl_2] (**29**) species of L-Met-Gly as the coordination of the amino and thioether donor functions.[264] The complex [Pt(L-ProGly)(DMSO)Cl] (**30**) also contained bidentate ligands with two nitrogen donors.[264] On the other hand, the platinum(II) complexes of Gly-L-His[264] and Gly-β-Ala[266] were described as tridentate coordination of the peptide molecules. The coordination of 3N donors was suggested in the complex [Pt(Gly-L-His)Cl] (**31**), while K[Pt(Gly-β-Ala)Cl] (**32**) was characterized by the (NH_2,N^-,COO^-) binding sites in fused five- and six-membered chelate rings.

The platinum(II)–ligand bond lengths have been compared with those reported earlier and the role of Pt–S bond in the toxicity of cisplatin has been discussed.[264] Several platinum(IV) complexes of dipeptides have also been pre-



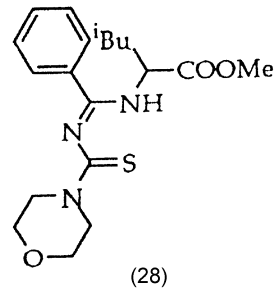
Scheme 3



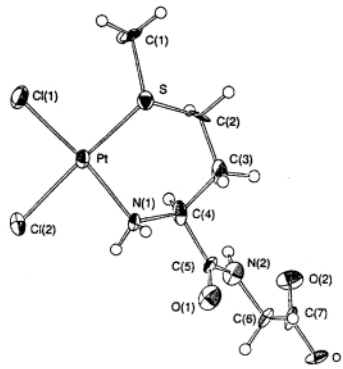
(27)

pared and structurally characterized by ^{195}Pt and ^{13}C NMR measurements. The biological activities of the complexes were also tested and selective inhibition of fungal cells was suggested.[265]

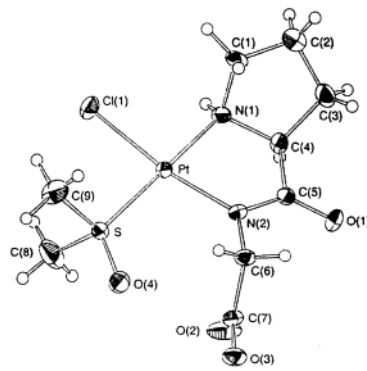
Compounds of vanadium are essential nutrients for higher animals, while in humans they show insulin-like properties. As a consequence, a huge number of vanadium complexes have been prepared in the past few years, including those of



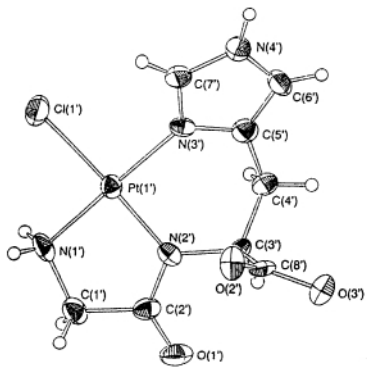
(28)



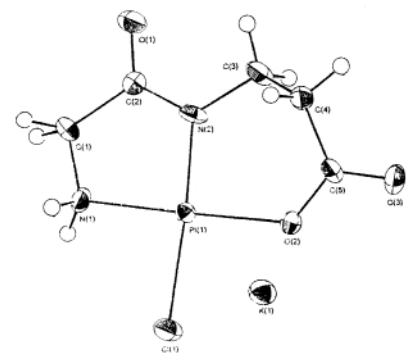
(29)



(30)



(31)



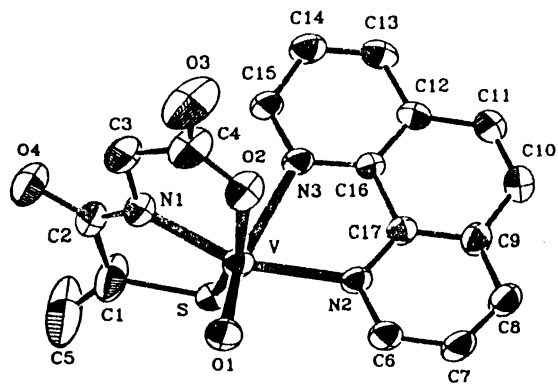
(32)

amino acids, peptides and related ligands. Schiff bases derived from the reaction of salicylaldehyde and simple dipeptides proved to be very efficient ligands to bind oxovanadium(IV), although the amide groups were not considered as metal binding sites.[269] The formation of a relatively stable adduct with the stoichiometry $Rb_2H_4V_{10}O_{28}$·2Gly-Gly·$2H_2O$ has been detected in the reaction of decavanadate ion with glycylglycine. A direct vanadium–peptide bond was not observed in this species and the peptide molecules interacted *via* hydrogen bonding with the polyanion.[270] Density functional theory computations were used to describe the binding modes between vanadate and glycylserine. In agreement with previous experimental findings, the coordination of the oxygen

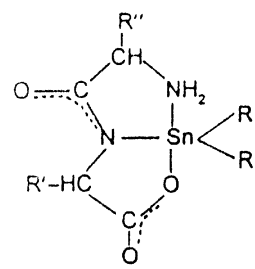
donors of the carboxylate or alcoholic functions was suggested in isomeric species.[271] It has been shown by several studies that glutathione as a complexing and reducing agent plays an important role in the biochemistry of vanadium. The oxovanadium(IV/V) complexes of sulfhydryl-containing pseudopeptides and dipeptides were studied recently to mimic the binding patterns of glutathione.[272,273] It is clear from these studies that ligation of vanadium(V) to glutathione is possible at physiological pH, which is then followed by the reduction to vanadium(IV). The resulting oxovanadium(IV) species form stable complexes with sulfur-containing peptides in which the deprotonated sulfur, amide and carboxylate functions are the major metal binding sites. This binding mode can be best represented by the solid state structure of the mixed ligand complex (**33**) containing an oxovanadium(VI) core and 1,10-phenanthroline and *N*-(2-mercaptopropionyl)glycine as ligands.[272,273]

New diorganotin(IV) complexes of several simple dipeptides have been prepared and characterized by various spectroscopic techniques.[274] The monomeric 1:1 complexes have distorted trigonal pipyramidal structure with *cis*-equatorial organic groups (**34**). Antimicrobial and antiinflammatory activities of the complexes have also been tested.

Metal complexes of peptides containing histidyl residues continue as the focus of interest. The peptides His-Pro-Gly-Ala-His and Pro-Gly-Ala-His were reac-



(33)



Where, R = n-Bu and Ph.

(34)

ted with various palladium(II) and platinum(II) complexes containing one or two free coordination sites (*e.g.* $[Pd(dien)]^{2+}$ and $[Pd(en)]^{2+}$). ^{1}H, ^{13}C and ^{195}Pt NMR measurements indicated that histidyl imidazole-N donor atoms are the major metal binding sites at any pH value. Monodentate binding of the imidazole residues resulted in the formation of N(1)- and N(3)-bonded isomeric species, but the imidazole-bridged dimeric and (NH_2,N(3))-chelate complexes were also detected.[275] Diorganotin(IV) complexes of L-Met-His have been synthesized and the crystal structure of $[Me_2Sn(Met\text{-}His)]\cdot 0.5MeOH$ determined by X-ray diffraction. Terminal amino, deprotonated amide and carboxylate functions were described as the metal binding sites, similarly to simple dipeptides (as shown by (**34**)) suggesting that neither the imidazole nor the thioether groups were involved in metal binding.[276] To investigate the binding modes of copper(II) in human prion proteins the terminally blocked octa- and tetra-peptides, Ac-HHGGGWGQ-NH_2 and Ac-HGGG-NH_2 respectively, have been synthesized and their complexes studied by CD and EPR spectroscopic techniques. It was found that the octapeptide had a unique metal ion coordination site containing a square pyramidal geometry with three amide nitrogen atoms and one oxygen atom at the edges of the equatorial plane and a further nitrogen atom apically linked.[277]

The abundance and outstanding biological significance of imidazole binding in metalloproteins resulted in the synthesis of many related ligands. Copper(II) complexes of *N*,*N*′-ethylene-bridged histidyl-tyrosine derivatives were prepared as models of galactose oxidase. Mononuclear, square pyramidal complexes were obtained in both the solid and solution states containing two histidine imidazole nitrogen and two phenolate oxygen atoms and a water molecule in the coordination sphere of the metal ion.[278] A trinucleating ligand derived from L-histidine containing two potentially tridentate amino-bis(imidazole) and one bidentate piperazine binding site has been synthesized recently. Stereochemical properties of copper(II) complexes were elucidated by means of UV-visible, CD and NMR measurements and a square-pyramidal geometry of the metal ion was suggested.[279]

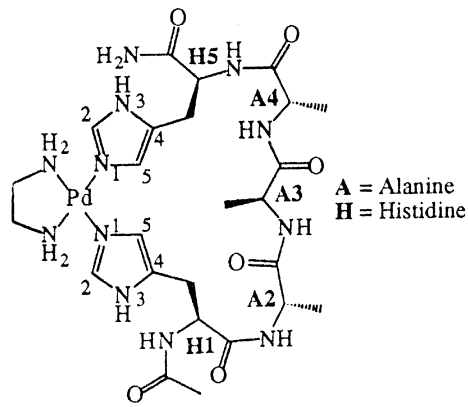
The biological significance of zinc finger peptides and metallothioneins have given a great impetus to further studies on the metal ion–sulfur coordination. As a consequence, large numbers of peptides containing both histidyl and cysteinyl residues have been synthesized and their complexes were obtained with different divalent metal ions.[280–283] The 34-residue peptide NZF-13 corresponding to the residues 543–577 of the NZF-1 protein and containing the CCHHC zinc binding domain was prepared and characterized.[280] It was found that the peptide binds cobalt(II) and presumably zinc(II) in a tetrahedral site *via* the three Cys and one of the His residues (His16). The replacement of His16 with Ala resulted in a structural rearrangement involving the metal binding of the other His residue. However, the replacement of both histidyl residues with alanine resulted in a dramatic change of properties indicating that at least one His is required for high-affinity metal binding. The nucleocapsid protein (NCP) from Mason-Pfizer monkey virus (MPMV) contains the evolutionary invariant Cys-X_2-Cys-X_4-His-X_4-Cys zinc finger structures. Metal complexes of 18-amino-acid peptides which

model the native zinc finger sequence have been synthesized and spectroscopically characterized. On the basis of the absorption spectra the cobalt(II) and cadmium(II) complexes of the peptides were described by regular tetrahedral coordination geometry. In contrast, nickel(II) complexes exhibit a single absorption band characteristic of square planar coordination geometry.[281] Electrospray mass spectrometric studies were performed on the zinc(II) complexes of zinc finger arrays, CCCC, CCHC and CCHH. It was found that two thiolate groups must be present to maintain the chelation with divalent zinc, while the other cysteine residues in the array retain thiol protons.[282] Spectroscopic monitoring of the metal binding in zinc finger peptides is a crucial point for the structural elucidation of these complexes. It has been reported that Ultraviolet Resonance Raman Sepctroscopy (UVRR) can be used for simultaneous monitoring of cysteine and histidine coordination and promise a wide range of applications.[283]

The CXXC amino acid sequence is found in a wide variety of metal binding proteins including MerP the mercury binding protein. The 3D structures and metal binding affinites of the sequence were compared in a 18-residue linear peptide and in the 72-residue protein in solution.[284] The binding affinity of the peptide was reduced by less than two orders of magnitude compared to MerP, but the order of affinities for different metal ions was similar for the two ligands. The chemistry of mercury(II) ions in biological fluids is dominated by the complexes formed with thiolate ligands. Hg(II)-substituted rubredoxin has been studied by various spectroscopic techniques and the formation of a $Hg(CysS)_4$ centre was observed in a slightly distorted tetrahedral environment. It is inferred furthermore that the formation of the thermodynamically stable HgS_4 centre is accompanied by rearrangements of the protein structure.[285] A synthetic tridecapeptide segment (49–61) representing the metal binding site of rabbit metallothionein has been prepared and reacted with cadmium(II) ions. The formation of a stable monomeric complex with 1:1 stoichiometry *via* thiolate coordination of four cysteine residues was observed. Whereas the thermodynamic stability of the peptide complex is relatively unperturbed, the kinetic reactivity is substantially enhanced as compared to the native protein.[286] The cadmium(II) binding properties of the C-terminal hexapeptide segment of mouse metallothionein I (Lys-Cys-Thr-Cys-Cys-Ala) were studied by CD, differential pulse polarography (DPP) and ^{113}Cd NMR methods. It was found that DPP was an excellent complementary technique to NMR and CD in the study of complexation of cadmium(II).[287] Iron(II), cobalt(II) and nickel(II) complexes of a 12-residue tetracysteinyl cyclopeptide have been investigated by EXAFS measurements. The formation of dimeric complexes was reported to contain distorted tetrahedral structure for iron(II) and cobalt(II) and distorted square planar stereochemistry for nickel(II).[288] Thiolate ligands generally form stable square planar complexes with divalent nickel(II) ion. However, an air-stable nickel(III) complex with $S_2N_2O_2$ chromophore has been isolated recently. The nitrogen donor atoms of this complex come from the deprotonated amide functions, which makes the coordination and redox chemistry of nickel-peptide complexes more versatile.[289] The interaction of $NaAsO_2$ with polypeptides containing internal Cys residues was monitored by HPLC and mass spectrometry.

The results demonstrate that arsenic(III) is able to distort the polypeptide structure in order to satisfy its desire to form trigonal-pyramidal thiolate coordination. It was suggested that similar interactions might be responsible for the toxicity of arsenic compounds.[290]

An important feature of the metal ion to peptide interaction is that it can stabilize specific peptide conformations in both solution and solid state. A stable, single α-helical turn (**35**) was formed in the reaction of $[Pd(en)]^{2+}$ with the pentapeptide Ac-HAAAH-NH_2. The 1:1 adduct is stabilized *via* the coordination of the terminal imidazole residues inducing α-helicity even in a pentapeptide.[291]



(35)

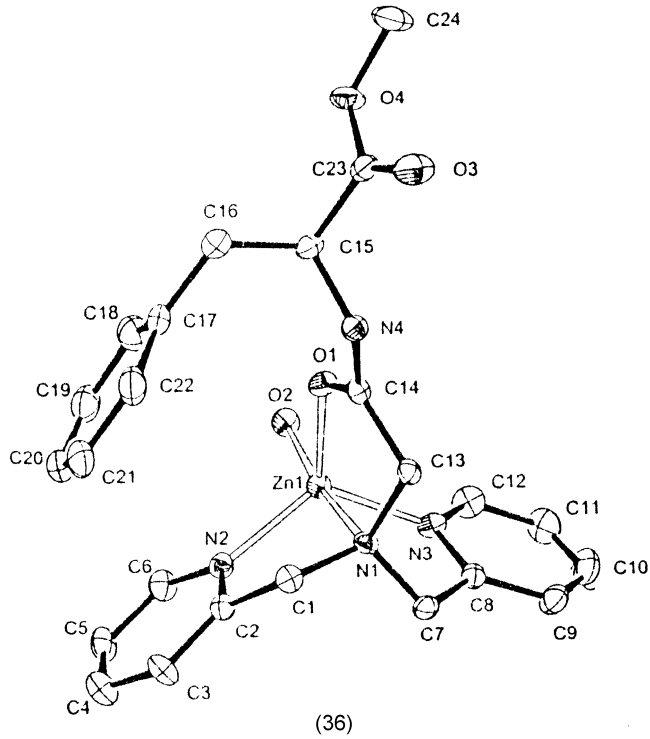
Metal ion coordination of oxytocin has been studied by electrospray mass spectrometry and rationalized by molecular mechanics simulation. Dramatic conformational changes were observed upon oxytocin coordinating to nickel(II), manganese(II) or palladium(II).[292] The interaction of calcium(II) ions with the peptide hormone melanostatin (Pro-Leu-Gly-NH_2) was followed by NMR studies. A 1:1 complex with the metal coordinated to the carbonyl moieties of Pro and Gly residues was shown to be the major species in solution. Upon metal complexation, substantial changes in the intrinsic chain flexibility of the peptide and in the exchange rates between water and amide protons were detected.[293] A series of amphiphilic peptides containing the dipeptide periodicity Asp-Leu or the tetrapeptide periodicity Leu-Asp-Asp-Leu has been synthesized. The peptides adopt a random coil conformation in pure water, while the addition of zinc(II) ions specifically induce a β-sheet structure for $(Asp\text{-}Leu)_n$ and α-helix for $(Leu\text{-}Asp\text{-}Asp\text{-}Leu)_n$-Asp. The addition of Mg^{2+} and NH_4^+ ions has no effect, whereas Ca^{2+} ion has only a slight effect on conformation.[294] Solution structure of the nickel(II) complex of the N-terminal pentadecapeptide of human protamine ($HP2_{1-15}$) was elucidated by NMR measurements and molecular modelling. A striking double-loop conformation was found, exhibiting the interactions of the aromatic ring of Tyr^8 residue with nickel(II) ion coordinated by Arg^1, Thr^2 and His^3 and the side chain of Arg^{15} residue. In this conformation all positively charged arginine side chains can locate on one side of the molecule providing a possibility for efficient contacts with DNA.[295,296] Circular dichroism

and NMR spectral methods were employed to study the conformation of the free and Ca^{2+}-bond forms of the peptides substance-P and its inactive analogue Ala^7-substance-P. The results show that both peptides assume a helical structure in non-polar solvents, but, compared with the native hormone, the N-terminus is less ordered in the model peptide. Addition of Ca^{2+} ions caused significant conformational changes in both peptides. However, while substance-P binds two Ca^{2+} ions in a cooperative manner, Ala^7-substance-P binds only one Ca^{2+} ion with a relatively weak affinity.[297] Mass spectrometric techniques were used to measure the lithium and sodium ion binding energies of N-acetyl and N-glycyl derivatives of 14 amino acids. Comparisons of basicities with metal binding energies indicate that the presence of a coordinating group (*e.g.* -OH or -COOH) in the amino acid side chain can significantly increase the lithium and sodium binding energies.[298]

It is widely accepted that in the case of small peptide molecules the formation of stable complexes is connected to the deprotonation and metal ion coordination of the amide functions of peptides. As a consequence, a great number of model compounds have been synthesized containing the amide and other ligating groups in chelating positions. The results obtained on the metal complexes of these ligands make a significant contribution to the coordination chemistry of peptides and can be used as structural models for metalloenzymes.[299–305] New chiral quadridentate ligands were synthesized in the coupling reactions of amino acids and bis(pycolyl)amine (bpa) and their zinc(II) complexes were structurally elucidated. The X-ray structure of $[(bpa\text{-}Ac\text{-}PheOMe)(H_2O)Zn]^{2+}$ cation (**36**) reveals a slightly distorted trigonal bipyramidal coordination environment and these types of molecules are promising candidates for enantioselective molecular recognition and hydrolytic studies.[299]

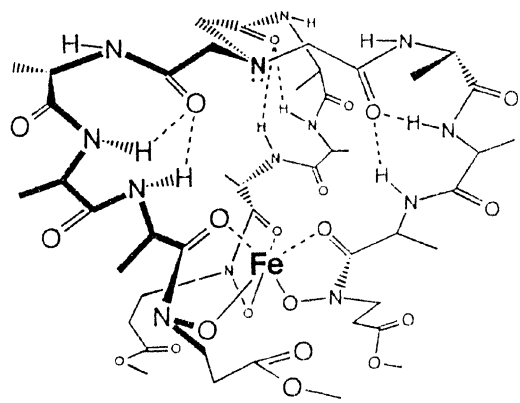
Some other derivatives of peptide or amide ligands whose metal complexes have been structurally characterized cover several different sulfonamides,[306–309] catechol-containing peptides[310] and Schiff bases.[311] The copper(II), cadmium(II), zinc(II) and lead(II) complexes of *N*-*p*-tolylsulfonyl-L-glutamic acid have been studied in both solution and solid state. Zinc(II) and cadmium(II) complexes were crystallized with 1:1 stoichiometry and only the oxygen donor atoms were present in the coordination sphere of the metal ions.[306] On the other hand, the metal ion promoted amide deprotonation and coordination were reported to occur in the alkaline solution of copper(II), cadmium(II) and lead(II). In the ternary systems containing 2,2′-bipyridine and the sulfonamide ligands, zinc(II) ions were also able to induce amide coordination.[307] Similar observations were obtained for the metal complexes of *N*-(2-nitrophenylsulfonyl)glycine, but the deprotonation and coordination of the amide functions were suggested for the cobalt(II) and nickel(II) complexes, too.[308]

Bleomycins are glycopeptide antitumour antibiotics and their biological activity depends on the presence of metal ions and molecular oxygen. The biochemistry and metal binding ability of bleomycins have been reviewed,[15] while the coordination chemistry of the cobalt(III)-bleomycin system has been investigated through NMR studies and molecular dynamics calculations.[312] A series of tripodal peptide hydroxamate ligands have been synthesized combining



(36)

H-(alanyl)$_n$-β-(OH)alanyl peptides (n = 1–3) with nitrilotriacetate. These ligands form six-coordinate octahedral complexes with iron(III) (**37**) in neutral aqueous solution. The stability and chirality of the complexes formed depend on the alanyl residues incorporated.[313]



(37)

3.2 Solution Equilibria – Speciation in Metal Ion–Peptide Systems. – Solution equilibria of the systems containing the most common transition elements and simple di-, tri- or tetra-peptides have already been well-characterized in the past

few decades. Most of these results come from potentiometric studies and the thermodynamic parameters and metal ion speciation are generally supported by independent spectroscopic measurements. The last few years, however, have brought about a significant improvement in the field of solution equilibria of peptide complexes, too. On the one hand, several new experimental techniques have been developed. These can be applied for the simultaneous monitoring of both metal ion speciation and solution structure of the peptide complexes. On the other hand, the improvement of computational methods and the more efficient synthetic procedures for the preparation of peptides have made it possible to study more and more oligopeptides containing a relatively high number of amino acid residues.

A series of multi-component EPR spectra of fluid aqueous solutions were used to characterize the metal ion speciation in various dipeptide complexes of copper(II).[314,315] The stability constants obtained in these studies showed a good agreement with the previous potentiometric results. The major advantage of the EPR analysis is, however, that it makes possible the detection of species present in very low concentration or the isomeric forms of various species. Copper(II) complexes of histidine-containing peptides were evaluated by the combined application of potentiometric and EPR measurements. It was concluded that EPR spectroscopy is an especially powerful technique, even at room temperature, for the characterisation of both metal ion speciation and binding sites of copper(II) complexes.[316] Visible absorption spectra of more than 100 copper(II) complexes, including many peptides, have been analysed and a good correlation between the spectral parameters and the metal binding sites was obtained. These data provide a good base for the elucidation of the solution spectra of multicomponent systems containing different N- and O-donor ligands.[317] A fluorescence study was performed to monitor the chelation between terbium(III) and Gly-Leu-Phe and related peptides in methanolic solutions. The lanthanide complexes showed relatively high thermodynamic stability and it was suggested that the complex formation reactions can be used to detect Gly-Leu-Phe and related ligands.[318] Isothermal calorimetric measurements (ITC) were used to determine the thermodynamic parameters of the nickel(II) and copper(II) complexes of histidine and its peptides, including bovine serum albumin. The experimental conditions and applicability of the method have been discussed in detail.[319]

Most of the solution studies in metallopeptide chemistry were devoted to the investigation of the role of side chain residues in complex formation processes. Stability constants of palladium(II) complexes of peptides containing only non-coordinating side chains have been reported recently.[320] Palladium(II) is one of the most effective metal ions to promote deprotonation and coordination of amide functions. The outstanding thermodynamic stability of these complexes, however, ruled out the pH-metric determination of reliable stability constants. An indirect potentiometric method, using chloride ions in high concentration as a competitive ligand, has been developed for the calculation of the metal ion speciation in palladium(II)-peptide systems. The $[PdH_{-1}L]$-type complexes, containing the tridentate (NH_2,N^-,COO^-)-coordinated ligands, were almost completely formed at pH 1 in the absence of chloride ion, while this process shifted to

the pH range 1–3 upon increasing chloride concentration. In the palladium(II)-dipeptide systems two different types of bis(ligand) complexes were detected by pH-metric and NMR studies: $[PdH_{-1}L_2]$ and $[PdH_{-2}L_2]$ containing tridentate/monodentate and bis(bidentate) coordinations, respectively. The pH range for the formation of the 4N-coordinated $[PdH_{-2}L_2]$ complexes was, however, very much influenced by the presence of the non-coordinating side chains. This means that the deprotonation of the second amide nitrogen occurred only in strongly alkaline solutions with the peptides containing side chains at the C-termini (*e.g.* Gly-Ala), whereas this process took place at physiological pH with C-terminal Gly residue (*e.g.* Ala-Gly). The palladium(II) complexes of tripeptides were characterized by the exclusive formation of the (NH_2,N^-,N^-,COO^-)-coordinated species $[PdH_{-2}L]$ at pH 4–5 in equimolar solutions. The presence of excess ligand, however, resulted in the formation of $[PdH_{-2}L_2]$ bis(ligand) complex and according to the NMR studies this species is a 4N complex with bidentate coordination of both ligands.[320] The effect of the C-terminal chelate ring size on the stability and coordination structure of copper(II) tripeptide complexes was studied by potentiometric, UV-visible and EPR spectroscopic methods. The binding mode of the major species $[CuH_{-2}L]$ was described by the common (NH_2,N^-,N^-,COO^-)-coordination if the chelate ring sizes were in the range from (5,5,5) to (5,5,7). The species $[CuH_{-1}L(OH)]$ was formed with 2N coordination, if the ring size of the C-terminal chelate exceeded the seven-membered ones.[321]

Potentiometric and spectroscopic studies were performed for the elucidation of the interaction of dimethyltin(IV) cation with several amino acids, peptides and related ligands. Complex formation was not detected with histamine and glycylhistamine containing only N-donors. However, the deprotonation and coordination of the amide functions were reported to occur in the dimethyltin(IV)-Gly-Gly and Gly-His systems. In these complexes, tridentate (NH_2,N^-,COO^-)-coordination of the dipeptides was suggested and the metal binding of the imidazole moiety was not observed in any cases.[322] The coordination properties of the dipeptides Gly-Asp and Asp-Gly towards dimethyltin(IV) cations have been investigated by potentiometric, NMR and Mössbauer spectroscopic techniques.[323] The metal ion coordination of the carboxylate functions was observed in the acidic pH range and it was the anchoring group for amide binding above pH 4. The binding mode of both dipeptide complexes was interpreted by the same structure as depicted by (**34**). In contrast with the previous findings the metal ion coordination of only oxygen donor atoms was suggested in the triphenyltin(IV) complexes of *N*-acetyl amino acids.[324] Dialkyltin(IV) and trimethyltin(IV) complexes of selected peptides were investigated by potentiometric method. The tridentate coordination of the ligands was proposed for the dialkyl metal centres, while, surprisingly, a monodentate coordination of the amino group was suggested for trimethyltin(IV) cation.[325]

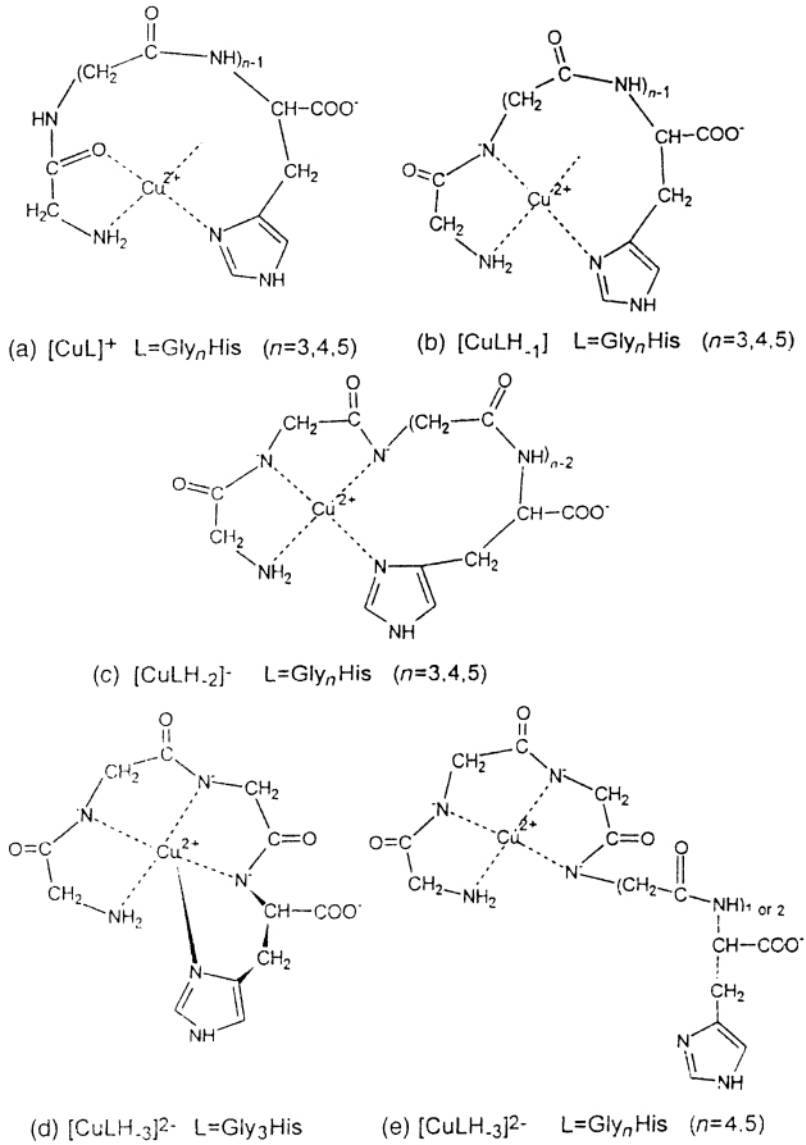
The structures and stability constants of the copper(II) complexes of dipeptides composed of N-terminal Arg, Lys or Gly and C-terminal Asp, Glu or Gly residues have been determined. Tridentate coordination *via* the terminal amino, deprotonated amide and α-carboxylate functions was characteristic of all ligands

in the major species $[CuH_{-1}L]$, except C-terminal Asp peptides, where the β-carboxylate of Asp was the metal binding site. The stability constants of the [CuL] and $[CuH_{-1}L]$ complexes were found to be in the order X-Asp $\geq$ X-Glu $>$ X-Gly (X = Arg, Lys, Gly), indicating that the C-terminal aspartyl residues enhances stability.[326]

α-Hydroxymethylserine (HmS) is a non-protein amino acid found in several antibiotic peptides. It has a CH_2–OH side chain at the α-carbon atom of serine and this modification has a very strong impact on the coordination chemistry of its peptides. Different metal complexes of the peptides containing HmS residues have been studied and it was found that the presence of the two alcoholic functions enhances the metal binding ability, without a direct involvement of the alcoholic-O donors in metal binding.[327–330] The results obtained for the tripeptides HmS-HmS-His-OH and HmS-HmS-His-NH_2 revealed that the latter ligand is the strongest Cu^{II} chelator among peptides due to steric shielding of the chelate plane as well as electronic effects.[327] Theoretical calculations and deprotonation microconstants indicated that the terminal $-NH_3^+$ group is more acidic than the imidazole nitrogen in HmS-HmS-His tripeptide. It distinctly changes the electron density within the amide bond which becomes a more effective donor atom than the regular amide functions of peptides. In the case of nickel(II) complexes the nickel(II)-catalysed oxidative decarboxylation of the ligand has also been observed similarly to other tripeptides containing C-terminal His residues.[328] Copper(II), nickel(II) and zinc(II) complexes of HmS-His were studied by potentiometric and various spectroscopic techniques. Tridentate, (NH_2,N^-,N(Im))-coordination of the ligand with all metal ions was suggested, but the stabilities of the complexes formed were significantly higher than those obtained for Gly-His or Ala-His.[329] In a more recent study the copper(II) complexes of deltakephalin (Tyr-(D)-Thr-Gly-Phe-Leu-Thr) and its analogues containing the HmS residue instead of one or both Thr residues have been investigated. The metal ion speciation of the three systems were found to be very similar, but the presence of HmS moiety resulted in a significant stabilization of the complexes.[330]

The investigation of the metal complexes of peptides containing histidyl residues has received increasing attention. Copper(II) complexes of the tetra-, penta- and hexa-peptides with His residue at the C-termini (Gly_3His, Gly_4His and Gly_5His) were studied by potentiometric and spectroscopic measurements.[331] The formation of the species $[CuHL]^{2+}$, $[CuL]^+$, $[CuH_{-1}L]$, $[CuH_{-2}L]^-$ and $[CuH_{-3}L]^{2-}$ was detected in all cases and their coordination modes are demonstrated by Scheme 4.

It is clear from Scheme 4 that the structures of the species $[CuL]^+$, $[CuH_{-1}L]$ and $[CuH_{-2}L]^-$ are determined by the formation of macrochelates in which the nitrogen donors of both N- and C-termini take part in metal binding. The stability of the macrochelates was reported to decrease upon increasing the length of the peptide molecule. The species $[CuH_{-3}L]^{2-}$ was described with the equatorial coordination of 4N donor atoms starting from the N-terminus, with a weak axial interaction of the imidazole residue.[331] The terminally blocked hexapeptide segment of histone H4 protein (Ac-Ala-Lys-Arg-His-Arg-Lys-NH_2) has



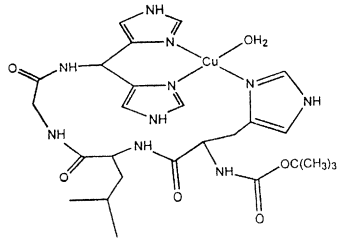
Scheme 4

been prepared and copper(II) and nickel(II) complexes studied by potentiometric and spectroscopic techniques. It was found that imidazole-N donor atom of His residue acts as the anchor for metal binding and a series of 4N complexes were formed at high pH values. The stability constants of these species were distinctly higher than those for similar peptides.[332] Oligopeptide fragments of pituitary adenylate cyclase activating polypeptide have been prepared and their complexation with copper(II) was studied.[333] The model peptides consisted of 5 to 14 amino acids and their sequence made it possible to study the effect of His residue

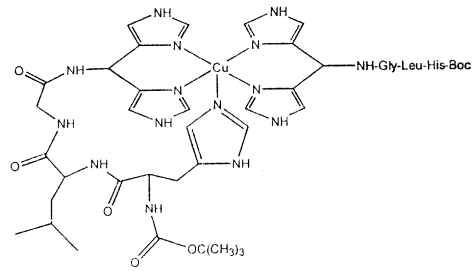
in the first and Asp residues in the second or third positions in the peptide chain. The formation of both monomeric and dimeric complexes was found with the pentapeptide containing N-terminal His residue, but dinuclear complex formation did not prevent the coordination of the second amide group at high pH. Asp residue in the third position stabilized the 3N species and prevented the coordination of the fourth nitrogen donor. The presence of Asp in the second position resulted in stability enhancement of the 2N complex, but it did not prevent further amide binding at high pH.[333] The tetrapeptide His-Gly-His-Gly contains two His residues and it results in a rather complicated equilibria of copper(II) complexes. The formation of dinuclear species was not observed at any pH, but the Raman spectra supported the involvement of two imidazole nitrogen atoms in metal binding. The deprotonation and coordination of all amide functions in strongly alkaline conditions was suggested.[334] Combined spectrophotometric and potentiometric studies were performed on the copper(II) complexes of human (Glu-Val-His-His-Gln-Lys-NH_2) and mouse (Glu-Val-Arg-His-Gln-Lys-NH_2) β-amyloid peptide segments. The replacement of the third His residue of the human peptide with Arg in the mouse peptide segment results in a significant change in the coordination chemistry of the ligands. The human peptide segment forms a very stable 4N complex in the pH range 4.5–10.5. The binding mode is very similar to those of any X-Y-His amino acid sequence and the histidyl residue present in the fourth position does not take part in metal binding. However, the His residue in the fourth position of the mouse peptide segment is the primary metal binding site of the molecule and forms a macrochelate with the N-termini. Acylation of the N-termini results in significant changes of both peptides.[335]

Imidazolyl residues are often linked covalently to amino acids and peptides to create potential model compounds for the active sites of metalloproteins. The chelating agents bis(imidazol-2-yl)methylamine (BIMA) and bis(imidazol-2-yl)propionic acid (BIP) were attached *via* amide bonds to the C- and N-termini of tripeptides respectively. The tripeptides contained one histidyl residue in all possible locations, while the other termini of the peptides were blocked.[336] The results obtained for the copper(II) complexes of these ligands revealed that the imidazole-N donor atoms of the bis(imidazolyl) residues were the major metal binding sites with all the ligands studied. The outstanding thermodynamic stability of the species $[CuL]^{2+}$ was explained by the equatorial coordination of the bis(imidazolyl) residue supported by the coordination of the side chain imidazole from the His residue as it is shown by (**38**) for the copper(II)-BOC-His-Leu-Gly-BIMA system. The bis(ligand) complexes $[CuL_2]^{2+}$ generally contained a five- or six-coordinated copper(II) ion with the bis(imidazolyl) nitrogen atoms in the equatorial plane and His imidazole-N atoms in axial positions as shown by (**39**).

Two new tripodal peptide ligands with histidine side chains have been prepared and their zinc(II) complexes were structurally characterized to mimic the binding sites of carbonic anhydrase. The coordination geometry of the complexes was similar to that of the enzyme and pK values of the coordinated water molecules were estimated.[337]



(38)



(39)

The side chain ε-amino groups of lysyl residues are also potential donor sites for metal binding in proteins. The amino acid lysine was present in many peptides already mentioned in this chapter, but the metal ion coordination of the ε-amino groups could not compete with the strong metal binder His or Asp residues. The results obtained for the copper(II) complexes of dipeptides of lysine (Gly-Lys, Ala-Lys, His-Lys and β-Ala-Lys), however, revealed that the ε-amino group can be an important bridging residue in dinuclear complexes.[338]

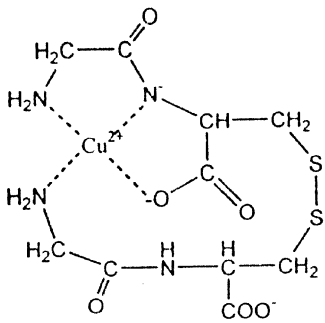
The roles of sulfur donor atoms in the metal binding of proteins have already been mentioned in Section 3.1. The redox activity and outstanding thermodynamic stability of several metal thiolate complexes, however, render the equilibrium studies of these ligands rather complicated. Glutathione is probably the most common and abundant naturally occurring tripeptide. Its coordination chemistry has been reviewed recently,[6] and the most important features of vanadium-glutathione interactions have also been summarised.[7] The results strongly support that vanadate can be easily transformed into oxovanadium(IV) by glutathione. The oxovanadium(IV) cation can interact either with the reduced or the oxidized forms of glutathione and the products depend on the metal ion to ligand ratio and pH of the solution.[7] The stability constants of the complexes formed in the reaction of dimethylthallium(III) with glutathione have been determined potentiometrically. The species $[(Me_2Tl)(GSH)]^-$ and $[Me_2Tl)GS]^{2-}$ were found in solution with a relatively low thermodynamic stability.[339]

Histones are among the most abundant proteins in cell nuclei and they are the potential targets for nickel binding and responsible for human carcinogenicity of nickel(II). The binding of nickel(II) to the core histone tetramer $(H_3–H_4)_2$ of chicken erythrocytes was followed by spectrophotometric titrations and conditional affinity constants were calculated. It was proposed that the binding of nickel(II) involved Cys^{110} and His^{113} residues of different H_3 subunits and the competition between histones and the low molecular weight chelators (*e.g.* histidine and glutathione) was discussed.[340] Cobalt(II) and zinc(II) binding properties of the N-terminal metal binding domain of HIV nucleocapside peptide (residues 1–18) have been determined and compared to those of related zinc(II) activated proteins.[341]

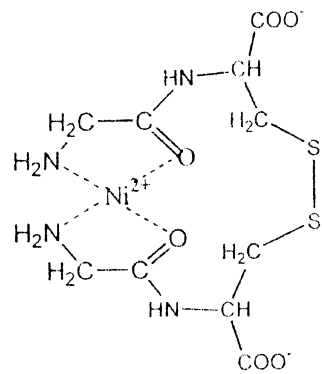
The interaction of lead(II) with human protamine (HP2) has been studied and its impact on the male reproductive systems discussed.[342,343] It was found that HP2 binds lead(II) at two different sites causing a conformational change in the protein. Thiol groups are the primary lead(II) binding sites. Affinity of HP2 for

lead(II) and zinc(II) binding was very similar, suggesting that lead(II) can compete with or replace zinc(II). A relatively intense lead(II)-thiolate charge transfer band was used to monitor lead(II) binding to the structural zinc(II) binding domains of proteins.[344] It was found that lead(II) ions are able to compete effectively with zinc(II) ions for Cys_4 sites under physiological conditions and the ratio of lead(II) to zinc(II) bound to a particular site is determined by the relative affinites of the two metals rather than being under kinetic control. Binding of lead(II), however, does not stabilize the correct fold in the peptides.[344]

Stability constants and structures of the complexes formed in the reaction of copper(II), nickel(II), zinc(II), cobalt(II) and cadmium(II) with L-cysteinylglycine disulfide, $(Cys\text{-}Gly)_2$, have been determined by potentiometric and various spectroscopic techniques.[345] Disulfide sulfur atoms were not metal binding sites in any of the systems studied although the amide and disulfide functions are in chelating positions in the molecule. Only copper(II) was able to promote deprotonation and coordination of amide functions. The high thermodynamic stability of the species $[CuH_{-1}L]$ was explained by the tridentate coordination of one side and a macrochelate from the other side of the molecule (**40**). In the case of the other metal ions the species [ML] predominated over a wide pH range and its outstanding thermodynamic stability was explained by the formation of a loop around the (NH_2,CO)-coordinated five-membered chelates in solution as in (**41**). The 1:1 complex $[Ni(Gly\text{-}Cys)_2H_2O]$ has been prepared in the solid state too, but its structure differs from that obtained in solution. The solid sample contained the same (NH_2,CO)-coordinated bis-chelates containing octahedral and coordinatively saturated (by the carboxylate residues) central metal ions in a polymeric network.[345]



(40)



(41)

Complex formation reactions of peptide derivatives were also studied and they included several cyclopeptides,[346] tetrazole derivatives,[347,348] amides,[349] Δ-peptides[350] and peptide–nucleic acid analogues.[351] Petallamides are cyclic octapeptides and their structure define, a macrocyclic cavity suitable for metal ion coordination under biological conditions. Zinc(II) complexes of the natural peptides and related model compounds have been studied by ^1H NMR and CD spectroscopies. It was found that the composition of the complexes depends on

the pH and the nature of the anion present in solution.[346] Tetrazole analogues of leucine-enkephalins have been newly synthesized and these ligands mimic the *cis*-amide bond conformation of enkephalins. The studies on the copper(II) complexes of the molecules revealed that the insertion of a tetrazole moiety into the peptide sequence considerably changes the coordination ability of the ligands. An unusually stable [$CuH_{-1}L$] species with 4N-coordination was formed, if the tetrazole moiety followed the third amino acid residue.[347] The tetrazole modified derivatives of the opiod peptides, β-casomorphins were also prepared and copper(II) complexes studied. The results demonstrated that the stability of copper(II) complexes can be significantly influenced by the conformation of the ligands and the highest stability was obtained for a 2N species containing the tetrazole unit in the position 3–4 in the peptide sequence.[348] Previous works have already indicated that a double bond placed between the α- and β-carbon atoms in an amino acid may have a critical impact on the coordination chemistry of the ligands. The metal binding abilities of di-, tri- and tetra-peptides containing α,β-dehydro amino acids (or Δ-peptides) were compared recently. The results obtained from combined potentiometric and spectroscopic studies revealed that the amide nitrogens of the Δ-amino acid residues are more effective in metal ion coordination than the parent analogues.[350] Peptide–nucleic acids (PNA) are synthetic analogues of DNA in which the natural phosphate–deoxyribose backbone is replaced by a peptide chain. The results obtained on the copper(II) and nickel(II) complexes of chiral peptide-nucleic acids that have thymine and adenine in their side chains revealed a significant extra-stabilization of various species. It was inferred that the specific stability enhancement can come from the interactions of the thymine and adenine residues with the equatorially coordinated metal ion, the peptide backbone or with an adjacent nucleic base.[351]

Mixed ligand complexes are promising models for the investigation of the biological activity and transport processes of metal ions. As a consequence, the ternary systems containing peptide ligands as one of the components were studied by many authors worldwide. The other components in these studies involved the complexation of taurine,[352] imidazole and derivatives,[353–356] dopamine and dopa[357] and various nucleosides and nucleotides,[358,359] Most of these systems were characterized by the stable equatorial coordination of the tridentate dipeptide ligands and the remaning coordination sites were saturated by the other ligands. In these ternary systems even the weakly coordinating monodentate ligands (*e.g.* taurine[352]) can compete with the hydrolytic reactions of the central metal ions. In another group of mixed ligand complexes a strongly coordinating bidentate ligand (*e.g.* bipyridyl or phenanthroline) competed with peptides for the coordination sites of the metal ions.[360,361]

3.3 Kinetics and Reactivity. – Only a few publications deal with the determination of the kinetic parameters of the complex formation reactions between peptides and metal ions. On the other hand, more and more examples prove that metal ions have a very significant effect on the reactivity of peptide molecules under physiological conditions. This subject has received increasing attention in

the last two years and, among others, the investigations include the metal ion promoted formation, hydrolysis, oxidation and degradation of peptide molecules.

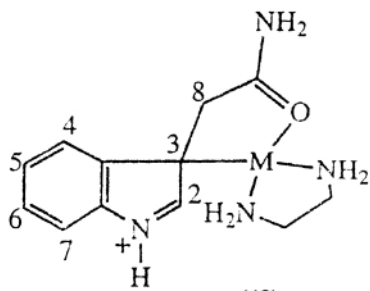
Stopped-flow spectrophotometric measurements were used to follow the kinetics of the cobalt(II) and zinc(II) binding to zinc finger peptides.[362] Cobalt(II) binding was demonstrated to be second order, first order in each peptide and cobalt(II) concentration. The displacement of cobalt(II) with zinc(II) was explained by dissociative mechanism with the rate of zinc(II) binding being substantially faster than cobalt(II) binding. The latter observation seems to be general for the cobalt(II)/zinc(II) substitution in both peptides and metalloenzymes. The aquation reaction of $[Pt(Gly\text{-}GlyH_{-1}Cl]^-$ has been studied by spectrophotometric measurements. A biphasic reaction mechanism was observed, the first step corresponds to the carboxylate ring opening of the tridentate (NH_2,N^-, COO^-)-coordination mode and the second one to the release of chloride ion.[363]

The most crucial prerequisites for the origin of life were the formation of peptides and proteins from amino acids under the conditions of the primitive earth. Metal ions could probably play a significant role in these processes and the observations available for the copper(II) promoted peptide synthesis have been reviewed.[11] Clays are known as minerals with a particular suitability for adsorption and catalytic processes. They were probably present on the primitive earth crust and could, therefore, have played a crucial role in the molecular evolution of bioorganic compounds. The catalytic activity of various clays in peptide bond formation was tested by the reactions of glycine, alanine and diglycine.[364] Mg-rich trioctahedral clays hectorite (smectite) and talc were found to be the most efficient catalysts. In another study the catalytic efficiency of clay, silica and alumina was compared in the reactions with a series of amino acids.[365] The reactivity of the amino acids decreased in the order: Gly > Ala > Pro ~ Val ~ Leu. The highest catalytic affinity was observed for alumina, the only catalyst producing oligopeptides in all systems investigated.

Selective cleavage of proteins is one of the most important procedures in biochemistry. The amide bond, however, is extremely unreactive. The uncatalysed hydrolysis of peptides under physiological conditions occurs with the half-lives of 250 to 600 years. Transition metal complexes are promising reagents for the cleavage of peptides and proteins. The palladium(II)-promoted hydrolysis of amide bonds in histidine- and/or methionine-containing peptides has been reported in several papers.[366–370] It was found that the complex $[PdCl_4]^{2-}$ regioselectively cleaves the amide bond involving the carboxylic group of histidine (*e.g.* the bond in His-Gly), while $[Pd(H_2O)_4]^{2+}$ cleaves, at comparable rates, the amide bonds involving either the carboxylate (His-Gly) or amino functions (Ac-His) of histidine. The rate constants for cleavage by $[Pd(H_2O)_4]^{2+}$ are approximately 10 times higher than those for cleavage by $[PdCl_4]^{2-}$. This study shows that kinetics and regioselectivity of the peptide cleavage may be controlled simply by choosing the ligands in the palladium(II) complexes.[366] The hydrolysis of the amide bonds in histidine-containing peptides was also promoted by chelated palladium(II) complexes (*e.g.* with bidentate amino acids), but no cleav-

age of Gly-His amide bond was observed in these reactions.[367,368] It was found that the rate of hydrolysis decreases as the steric hindrance of the palladium(II) chelates increases: en > 1,2-pn > Meen > *S*-methyl-L-cysteine > Cys > Met > Me_4en.[368] On the other hand, the stable tridentate coordination of histidine containing peptides (*e.g.* Ala-His or Ser-His) was found to be unfavourable for the hydrolytic cleavage of peptide bonds.[369]

Tryptophan has much lower affinity to palladium(II) than methionine or histidine. Nevertheless, it has been reported recently that palladium(II) regioselectively cleaves the amide bond on the C-terminal side of tryptophan.[371,372] Unlike histidine peptides, the reaction was reported to occur only in non-aqueous media and it was explained by an unusual coordination of the indolyl residue forming a Pd–C bond (**42**). Various palladium(II) complexes were also used to cleave macromolecules including oxidized insulin and horse heart cytochrome c. In the case of insulin[373] the reaction was regioselective and followed first order kinetics with a half-life of 4.8 days at 40 °C. Native cytochrome c and apo-cytochrome c have quite different conformations and sulfur atoms for palladium(II) binding, but the site (His^{18}-Thr^{19}) and the yield of cleavage were comparable.[374]

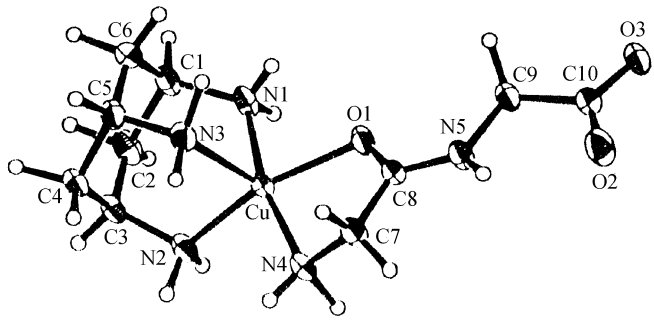


M is Pt(II) or Pd(II)

(42)

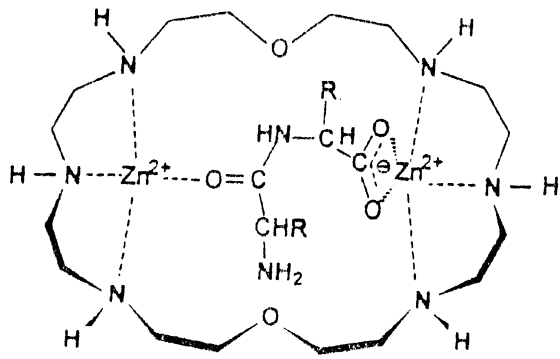
In addition to the palladium(II) complexes several other metal ions and complexes were reported to promote hydrolysis of amide bonds. Pentacoordinated zinc(II) and cadmium(II) complexes containing thioether sulfur and nitrogen donor atoms have been prepared and found to be active in amide cleavage.[375] The existence of a five-coordinated copper(II) complex was reported as an intermediate in the copper(II)-catalysed hydrolysis of Gly-Gly to glycine.[376] The structure of the intermediate species containing a tridentate ligand (*cis*,*cis*-1,3,5,-triaminocyclohexane) and (NH_2,CO)-coordinated Gly-Gly molecule is shown by (**43**). It was suggested that the metal ion coordination of the oxygen atom activates the carbonyl group and it is then attacked by external hydroxide ions.[376]

OBISDIEN (1,4,7,13,16,19-hexaaza-10,22-dioxacyclotetracosane) is a bis-chelating macrocyclic ligand and forms dinuclear complexes with copper(II) and zinc(II) *via* the coordination of three nitrogen donor atoms. It was found that the zinc(II) complex of OBISDIEN catalysed the hydrolysis and deuteration reactions of Gly-Gly.[377] The catalytic activity of the dinuclear complex was explained



(43)

by the formation of an active intermediate as shown by (**44**). It has been reported recently that cerium(IV) ions efficiently catalyses the hydrolysis of oligopeptides under mild conditions.[378] The half-life of amide linkages was only a few hours at 50 °C. In the case of tripeptides the amide linkage near the N-terminus was hydrolysed preferentially and the reaction was especially fast when the substrates had no metal binding side chains. The hydrolytic reaction was proposed to proceed *via* the coordination of both terminal amino and carboxylate functions. The catalytic activity of cerium(IV) was far greater than those of other lanthanides and oxidation cleavage was not observed under reaction conditions.



(44)

The investigation of the various redox reactions represents another subject of interest in the field of metal ion-promoted transformations of amino acids and peptides. The results obtained from electrospray mass spectrometric and X-ray diffraction studies provide clear evidence that a copper(II)-peroxide adduct with η^1-coordination mode can cleave the C–N bonds in peptides and hydroxylate the alkyl group nearby.[379] It was shown in another study that ruthenium(III) ions can efficiently catalyse the oxidation of peptide backbone without its fragmentation.[380] The catalytic oxidation of Ac-Gly-Ala-OEt was performed with peracetic acid in the presence of $RuCl_3$ and gave the α-ketoamides Ac-NH-COCO-AlaOEt and ethylpyruvate, obtained by the oxidations of the C^{α}-positions of Gly and Ala residues respectively. The oxidation reactions of cyclic His peptides was

monitored in the presence of ascorbate/Cu(II)/O_2 system.[381] H_2O_2 and superoxide anion were deduced to be the reactive intermediates in the reaction and it has been shown that the oxidation of these peptides leads to 2-oxo-His as the major degradation product. The role of His residues was also demonstrated in the nickel(II)-induced cleavage and oxidation damage of histones.[382] Nickel(II)-dependent hydrolysis of the Glu^{121}-Ser^{122} peptide bond was reported to occur in the synthetic 34-mer model peptide representing the entire C-terminal tail of the major human H2A histone variants. The presence of His^{124} in the peptide seems to be crucial for the hydrolytic reaction. It was also shown that nickel(II) bond in the hydrolytic product SHHKAKGK is capable of promoting oxidative degradation.

The reactions of copper ions with D-penicillamine have been monitored by ^{1}H NMR spectroscopy in the presence and absence of glutathione under aerobic as well as anaerobic conditions. It was suggested that the formation of the stable mixed valence cluster species of copper(II) and penicillamine is not affected by the presence of glutathione, which can be the reason for the therapeutical applications of D-penicillamine.[383] Most evidence to date indicates that physiological activity of platinum(IV) complexes is related to their reduction to platinum(II) by potential cellular reductants including glutathione. The reductions of various platinum(IV) complexes with glutathione were investigated using stopped-flow spectrophotometric measurements. The redox reactions followed the second-order rate law and the thiolate species were the major reductant under the reaction conditions used. The kinetic results together with literature data indicate that platinum(IV) complexes with *trans*-Cl–Pt–Cl axis are reduced rapidly (within 1 s) by glutathione as well as ascorbate.[384] The kinetic parameters of the reaction between superoxo chromium(III) (CrO_2^{2+}) and glutathione have been determined spectrophotometrically.[385] It was suggested that the real oxidant for glutathione is not the superoxo species, but the oxochromium(IV) cation CrO^{2+}.

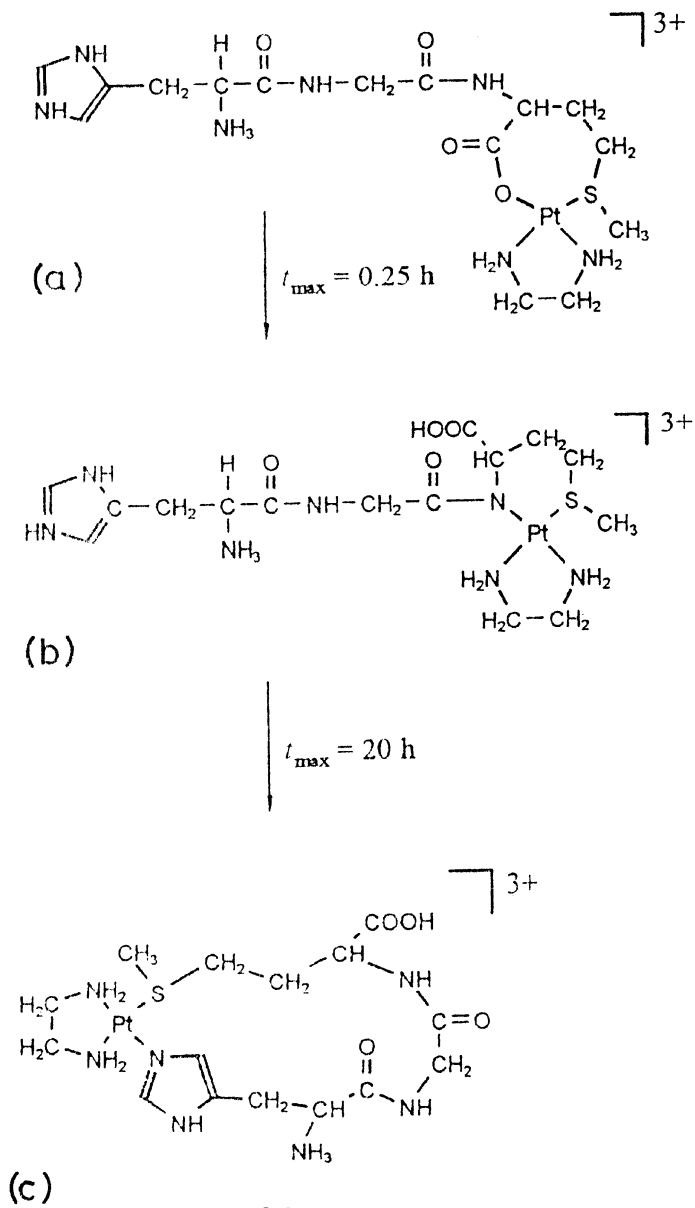
Cisplatin is a widely used anticancer drug and its activity arises from its ability to bind the N(7) position of guanine in DNA. The high affinity of platinum compounds for sulfur coordination and the great abundance of the corresponding biomolecules in the cell have raised the question of whether Pt–S bonded adducts might play a role as intermediates in DNA platination. The intramolecular migration of $[Pt(dien)]^{2+}$ from methionine-S to imidazole-N in the peptides His-Gly-Met and Ac-His-Ala-Ala-Ala-Met-NHPh was studied by HPLC and NMR spectroscopy.[386] The thioether-bonded complexes were formed in relatively fast reactions followed by slow intramolecular migration to the nitrogen donor atoms of histidyl residues. Both N(1) and N(3) donors of imidazole were considered as metal binding sites, but with a high preference for the N(1) binding. The studies on the reaction of DNA oligonucleotides and *S*-methylated glutathione with $[Pt(dien)]^{2+}$ provided further evidence for the kinetic preference of sulfur coordination and its substitution by oligonucleotides. The rates at which such substitution occur, however, make it improbable that these reactions significantly contribute to the antitumour activity of cisplatin and related compounds.[387] The pH and time dependent reactions of the bifunc-

tional $[Pt(en)(H_2O)_2]^{2+}$ with tri-, tetra- and penta-peptides containing terminal Met and His side chains were studied by chromatographic and NMR techniques. The thioether residue worked as an anchor for metal binding in the form of a seven-membered (*S*,*O*)-chelate (see Scheme 5a) and it was followed by a relatively rapid metallation of the neigbouring amide nitrogen for all three peptides at pH < 4 (Scheme 5b). After reaching a concentration maximum within 10–50 hours, the six-membered [*S*,*N*(amid)]-chelate slowly isomerised to the thermodynamically preferred [*S*,*N*(imidazole)]-macrochelate (Scheme 5c).[388]

Thermodynamic, structural and kinetic studies were performed in the mixed ligand complexes of palladium(II) with various thioether ligands.[389] Palladium(II) complexes are frequently used as structural probes of platinum(II), because the much faster formation kinetics of palladium(II) complexes makes the determination of both kinetic and thermodynamic parameters easier. In agreement with the previous findings on platinum(II) complexes, the kinetic preference for thioether binding and thermodynamic preference for the coordination of N-donor ligands were reported for the palladium(II) complexes, too. The quantitative data made it possible to calculate time dependent speciation curves of the ternary systems. At the same time, the stability constants obtained for the thioether complexes revealed that the selectivity of palladium(II) for thioether binding can be significantly influenced by the other donor atoms present in the coordination sphere of the metal ion. $[Pd(terpy)]^{2+}$ and $[Pd(Gly\text{-}MetH_{-1})]$ containing aromatic-N and thioether-S donor atoms, respectively, had the lowest affinity for thioether binding and it was explained by steric and electronic effects.[389] A similar study has been performed on the reaction of $[Pt(dien)Cl]^+$ and [Pt(Gly-Met)Cl] with Ac-Met-His. The formation of the Pt–S bond was kinetically favoured in this case too, but the migration to imidazole-N donors was observed only for $[Pt(dien)Cl]^+$. This observation was explained by the decreased kinetic lability and enhanced stability of the thioether adduct of [Pt(GlyMet)Cl], but quantitative kinetic parameters and stability constants were not reported.[390]

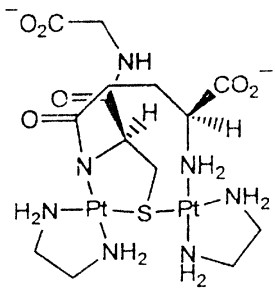
In addition to the thioether group of methionine, the thiol and disulfide functions of reduced and oxidized glutathione are also possible binding sites of platinum(II) complexes. An unusual glutathione-bridged diplatinum(II) macrochelate (**45**) was isolated in the reaction of $[Pt(en)Cl_2]$ either with reduced or oxidized glutathione. Preliminary HPLC and NMR studies revealed that the sulfur-bridged dimer can react with GMP and it may have a significant role in the biological activity of cisplatin. On the other hand, the formation of the thiolate-bridged complex in the reaction of $[Pt(en)Cl_2]$ with oxidized glutathione suggests that platinum(II) can be an efficient catalyst for the cleavage of disulfide bonds under mild conditions.[391]

A histidine-2′-deoxyguanosine hybrid molecule (**46**) has been synthesized to compare the platinum(II) binding affinites of imidazole and guanine nitrogen donor atoms. The reaction of (**46**) with cisplatin resulted in the formation of two isomers containing N(7) of guanine and N(1) or N(3) of imidazole as the metal binding sites. No preference of the metal for either of these N-donors was observed.[392]

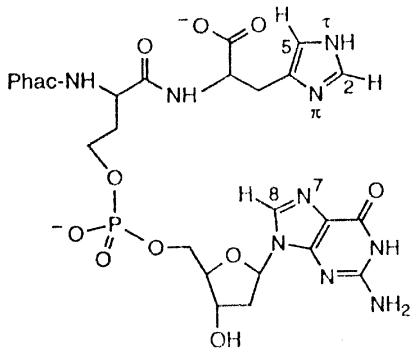


Scheme 5

An interesting field in the coordination chemistry of peptides is the use of peptide complexes themselves as efficient catalysts in many redox processes. A variety of copper(II) complexes with di-, tri- and tetra-peptides containing glycyl and histidyl residues have been investigated by EPR spectroscopy and their superoxide scavanger activity tested.[393] It was inferred that SOD activity increases with the increase of the equatorial distortion of copper(II) complexes. Complexes of glycine showed the highest activity, but comparable values were



(45)



(46)

obtained for oligoglycines. Histidine-containing peptides were less active, showing a reproducible increasing trend in SOD activity from GGHG to HGG. Copper(II) complexes of oligopeptides of histidine [$(His)_l Gly$ ($I = 4, 9, 19, 29$)] were used to catalyse the autooxidation of ascorbic acid. The peptide complexes ($i \geqslant 9$) enhanced the reaction approximately two-fold in relation to aqueous copper(II). The catalytically active copper(II) was accumulated in an imidazole cluster composed of at least six histidyl residues, while the other copper(II) which was complexed tightly with the terminal NH_2-X-Y-His moiety inhibited the autooxidation.[394] The catalytic activity of the copper(II) complex of carnosine has also been investigated and found to catalyse the hydrolysis of amino acid esters.[395]

The nickel(II) and copper(II) mediated DNA oxidation by H_2O_2 was studied in the presence and absence of human protamine HP2 having a strong metal binding motif, Arg-Thr-His, at the N-termini. Aqua complexes of nickel(II) and copper(II) were found to promote oxidative DNA strand scission and base damage, but copper(II) was more effective than nickel(II). In contrast, both metal ions formed complexes with the pentadecapeptide segment $HP2_{1-15}$, but only the copper(II) catalysed destruction of DNA strands were prevented by the pentadecapeptide.[396] Five peptides containing the (His-X_2)His or (His-X_3)His motifs have been synthesized and copper(II) complexes tested as catalysts in the oxidation of ascorbate and dismutation of superoxide anion. The catalytic efficiency of the model peptide complexes was much lower than that of the enzyme ascorbate

oxidase, but two of the complexes showed rather high activity for dismutation of superoxide.[397]

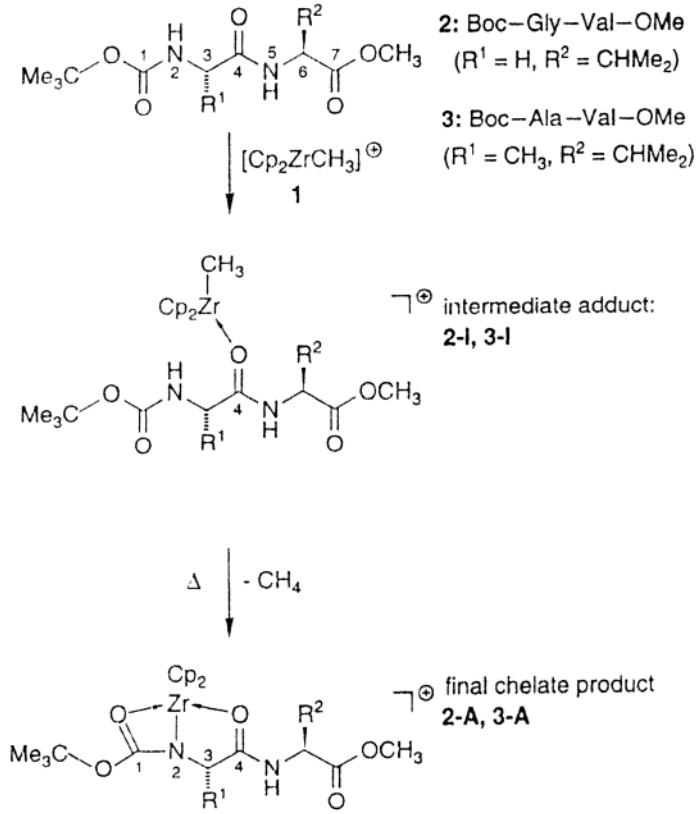
A series of rhodium(I) and iridium(I) complexes with multidentate C_2-symmetry ligands containing the amide functions has been synthesized and found to be efficient catalysts for the hydrogenation of olefins.[398] The stereoselective properties of oxygenated diastereomeric dipeptide systems were also studied during the uptake of molecular oxygen by cobalt(II) chelates.[399]

3.4 Synthetic, Analytical and Biomedical Applications of Peptide Complexes. – One of the major developments in the field of metallopeptide chemistry is reflected in the increasing number of publications dealing with the possible applications of peptide complexes. Some of these applications have already been summarized in review papers and they were cited in the introductory part of this chapter.[9–11,14,16,17]

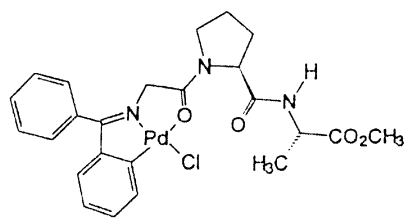
The investigations in the field of bioorganometallic chemistry represent one of the major uses of peptide complexes. Bioorganometallic chemistry attempts to combine favourably the characteristic features of typical biomolecules (such as amino acids and peptides) with those of organometallic building blocks in order to generate new reagents for organic synthesis, selective catalysis or analytical purposes. Several papers have been published on the preparation and reactivity of ferrocenoyl peptides and related molecules which can be used as redox markers and synthetic probes or for the selective monitoring of bio-molecules.[400–404] The ferrocene group is a selective electrochemical probe and can be applied to detect structural changes, because the redox potential of the ferrocenyl group is influenced by the amino acid or peptide residues.[400] An interesting coordination behaviour of the methylzirconocene cation ($[Cp_2ZrCH_3^+]$) was observed in its reaction with N- and C-terminally protected peptides. The strongly electrophillic methylzirconocene cation reacted readily with the carbonyl oxygen functions of the N-terminal amino acid residues and after a thermally induced methane elimination it was followed by the formation of a stable chelate involving the amide-N donor atoms (Scheme 6).[404]

Another group of studies in the field of bioorganometallic chemistry was devoted to the structural characterisation of various palladium(II) complexes.[405–407] The cyclopalladated compounds containing Pd–C bonds (**47**) are especially interesting and they can be obtained from the reactions of $[PdCl_4]^{2-}$ with Schiff bases or peptides.[405,406]

The biomedical applications of peptides as effective ligands for radiolabelled molecules or imaging agents received special interest. ^{99m}Tc is probably the most commonly used radioactive isotope in medical imaging, while the chemically related ^{186}Re/^{188}Re isotopes are promising candidates for internal radiotherapy. As a consequence, technetium and rhenium complexes of peptides and derivatives were studied by several research groups.[408–419] It is not the aim of this review to give a general survey of these biomedical investigations, but it should be emphasized that radiolabelled peptide complexes have a great potential as radiopharmaceuticals, because they offer high target specificity and a great deal of flexibility in their design. The peptides of cysteine and other sulfur containing



Scheme 6

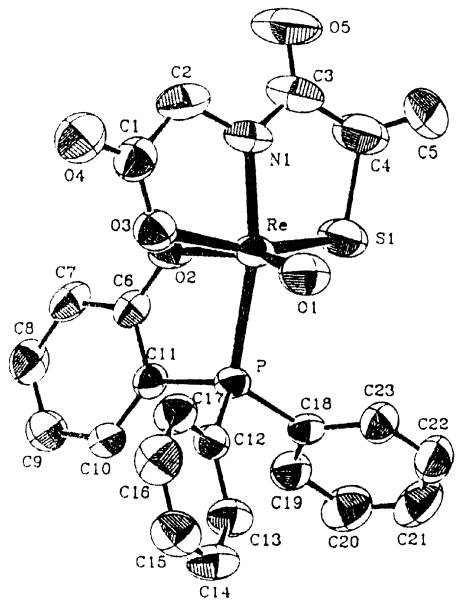


(47)

ligands are probably the most frequently used ligands in these applications,[408–416] but various mixed ligand systems are successfully applied, too.[414,415] The 3D structure of a mixed ligand oxorhenium(V) complex containing the bidentate *o*-diphenylphosphino-phenolato ligand and the tridentate peptide fragment, tiopronin, is shown in (**48**). The high stability of these oxorhenium(V) complexes was put down to the formation of closed-shell 18-electron octahedral structure and thiolate coordination.[408,415] Somatostatin was reported to show inhibitory effects on a wide range of tumours, so a number of conjugates of somatostatin-containing macrocyclic or multidentate chelators have been prepared and metal complexes characterized.[418–420] In a similar way, the DTPA-neuropeptide conju-

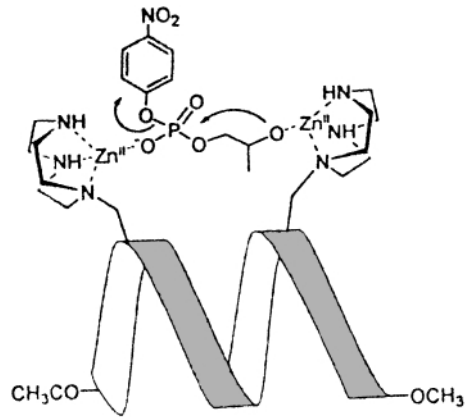
gates can be used for imaging brain amyloid responsible for Alzheimer's disease.[421]

An important development in the coordination chemistry of peptides is that peptide complexes are frequently attached to other biomolecules and the resulting species can be used as sensors or markers for analytical and biomedical purposes. The synthesis of a tripeptide conjugate of cisplatin has been reported and it was suggested that similar species can be used to overcome the tumor resistance of platinum drugs.[422] In another report the preparation of cyclopeptide-metal complex conjugates has been described using the reaction between linear peptides of cysteine and $[Pt(terpy)]^{2+}$. The high affinity of platinum for sulfur binding was used in this strategy and it was proposed that complexes of $[Pt(terpy)]^{2+}$ can be considered as both protecting and promoting groups for peptide cyclisation.[423] An effective transphosphorylation catalyst containing zinc(II) was obtained with the conjugation of a heptapeptide and two triazacyclononane macrocycles. A clear indication of cooperativity of the zinc(II) binding sites was observed in the catalytic cycle and it was explained by the existence of an intermediate (**49**) formed in the reaction of the substrate and the dinuclear complex.[424]



(48)

It has already been demonstrated that the nickel(II) chelate of nitrilotriacetate selectively binds proteins containing stretches of consecutive histidine residues. The binding strength of this type of coordination has been studied and the potential applications discussed.[425] Histidyl residues of polypeptides and proteins play an important role in some chromatographic techniques. Immobilized metal ion affinity chromatography (IMAC) is a method used in the separation of proteins with one or more exposed histidine residues. A commonly used IMAC



(49)

column has copper(II) sites supported by chelation that is attached to the resin. The coordination of histidine-containing hexapeptides towards copper(II) and palladium(II) complexes of *N*-methyliminodiacetate has been studied by spectroscopic techniques as potential models for IMAC binding sites.[426,427] Diaminodiamide ligands were prepared in the reaction of chiral amino acids and diamines. Solution equilibria and solid state structure of the complexes have been elucidated and the results provide valuable clues to the understanding of the mechanism of enantiomeric separation.[428] Five-coordinate trigonal-bipyramidal mixed ligand complexes of palladium(II) have been prepared with cysteine and glutathione (A ligands) and tris(2-(diphenylphosphino)-ethyl)phosphine (B ligand). The high reactivity for thiolate coordination in the axial position has been confirmed and used for the separation of L-cysteine from other amino acids and selective determination of reduced glutathione.[429] A new amperometric biosensor based upon a bilayer from glutathione sulfhydryl oxidase and osmium-polyvinyl-pyridine gel has been developed and used for the detection of the oxidized and reduced forms of glutathione.[430]

Artificial peptides containing synthetic metal binding sites can be promising models for the design of metal ion-assisted semisynthetic enzymes.[431–433] Ribonuclease-S′ bearing iminodiacetate as a metal binding site was designed and CD spectroscopy confirmed that copper(II) ion induces a change in the α-helix conformation of the chemically modified peptides.[431] A series of metallopeptides has been designed on the basis of the term 'metal ion induced distinctive array of structures' (MIDAS).[432] These peptides contain cysteine residues, and the sulfhydryl groups work as an anchor for the binding of oxorhenium(V) cores. The complexes show an excellent structural diversity and specificity in inhibiting human neutrophil elastase.

Poly(amino acid)s are of interest for modelling proteins and they may have several important applications including water soluble biopolymers and selective membranes. The coordination crosslink between adjacent polymer chains can be created by various metal ions. The interaction of several first row transition elements with poly(L-histidine)[434] and poly(L-lysine)[435] was studied in

the solid state and the effects of different metal ions are discussed. Peptide nucleic acids (PNA) constitute a relatively young class of DNA analogues with promising biological applications. For analytical monitoring it is desirable to attach PNA to an independent and sensitive spectroscopic probe. Synthesis and full characterization of transition metal derivatives of T-PNA monomers and oligomers including ferrocene-PNA, chromiumtricarbonyl-PNA and bipyridylruthenium-PNA have been reported and possible applications discussed.[436,437] The selective modification of DNA and RNA by transition metal complexes represents an increasing area of research in bioinorganic chemistry. Studies on new, water-soluble Ni(salen)-type complexes with DNA have been carried out under oxidative conditions. The results point to an efficient coupling process and are compared to that of nickel(II)-peptide complexes.[438]

Ethidium bromide is an organic dye producing induced circular dichroism (ICD) upon binding to DNA. The synthesis of an ethidium–peptide conjugate displaying metal dependent ICD has been reported recently.[439] The results show that binding of zinc(II) results in very significant structural changes in the peptide conjugate, which are sensitive to changes of external parameters, *e.g.* temperature and pH of the samples.

The nature of the interaction of metalloporphyrins with peptides has been a matter of interest for several decades. To probe the importance of hydrophobic interactions between peptides and the porphyrin face, the effects of multiple alanine residues were examined. Histidine was utilized as a ligating residue and it was suggested that hydrophobic interactions can be the major components in the formation and stability of heme–peptide complexes.[440] The interaction of octaethylporphynato iron(III) and gallium(III) complexes with the invariant peptide fragment containing Cys residues in the active site of cytochrome P-450 was studied by several spectroscopic techniques. The formation of an NH $\cdots$ S hydrogen bond was observed with both metal ions and it contributed to the stabilization of the high spin iron(III) resting state.[441]

Microorganisms such as bacteria and yeasts form CdS to detoxify toxic cadmium(II) ions. It has been shown that cysteine and its peptides including glutathione and phytochelatins can be used *in vitro* to dictate the formation of discrete sizes of CdS and ZnS nanocrystals. The diameter of the ZnS-glutathione nanocrystals was 3.45 ± 0.5 nm in a hexagonal geometry.[442] The reaction of $[RuCl_3(NO)(H_2O)_2]$ with the tripeptides GGG and GGH was studied to obtain information on the biological transport of nitrosyl complexes. In biomedicine ruthenium complexes are considered as both delivery agents to provide NO and scavangers of NO. It was found that triglycine is coordinated to ruthenium(III) in a non-labile complex *via* the terminal amino and the adjacent amide functions. As opposed to this, monodentate imidazole coordination of GGH was reported to predominate under the same conditions and this species was suggested as carrier of $[Ru(NO)Cl_3]$ under cellular conditions.[443]

4 References

1. *Chemical Abstracts Selects on Amino Acids, Peptides and Proteins*, published by the American Chemical Society and Chemical Abstracts Service, Columbus, Ohio.
2. P. Tsiveriotis and N. Hadjiliadis, *Coord. Chem. Rev.*, 1999, **190–192**, 171.
3. P. Tsiveriotis, G. Malandrinos and N. Hadjiliadis, *Rev. Inorg. Chem.*, 2000, **4**, 305.
4. L.F. Krylova, *Khim. Interesakh. Ustoich. Razvit.*, 1999, **7**, 465.
5. H. Kozlowski, W. Bal, M. Dyba and T. Kowalik-Jankowska, *Coord. Chem. Rev.*, 1999, **184**, 319.
6. A. Krezel and W. Bal, *Acta Biochim. Pol.*, 1999, **46**, 567.
7. E.J. Baran, *J. Inorg. Biochem.*, 2000, **80**, 1.
8. T. Kiss and I. Lázár, *Aminophosphonic Aminophosphinic Acids*, V.P. Kukhar and H.R. Hudson, eds., J. Wiley and Sons Ltd, Chichester, UK, 2000, pp. 285–325.
9. R.J. Browne, D.A. Buckingham, C.R. Clark and P.A. Sutton, *Adv. Inorg. Chem.*, 2000, **49**, 307.
10. G.T. Crisp, *Amino Acid Deriv.*, G.C. Barrett, ed., Oxford University Press, Oxford, UK, 1999, pp. 145–183.
11. B.M. Rode and Y. Suwannachot, *Coord. Chem. Rev.*, 1999, **190–192,** 1085.
12. M. Nagaoka and Y. Sugiura, *J. Inorg. Biochem.*, 2000, **82**, 57.
13. M.D. Harrison, C.E. Jones and C.T. Dameron, *J. Biol. Inorg. Chem.*, 1999, **4**, 145.
14. K. Kikuchi, R. Ansai and K. Tanaka, *Seibunkaisei Kemikarusu to Purasuchikku*, K. Tomita, ed., Shi Emu Shi, Tokyo, 2000, p. 1945.
15. D.L. Boger and H. Cai, *Angew. Chem. Int. Ed.*, 1999, **38**, 448.
16. L. Thunus and R. Lejeune, *Coord. Chem. Reviews*, 1999, **184**, 125.
17. D.E. Reichert, J.S. Lewis and C.J. Anderson, *Coord. Chem. Reviews*, 1999, **184**, 3.
18. W. Bal, H. Kozlowski and K.S. Kasprzak, *J. Inorg. Biochem.*, 2000, **79**, 213.
19. M. Shionoya and K. Tanaka, *Bull. Chem. Soc. Jpn*, 2000, **73**, 1945.
20. D. Wen, Y. Liu and S. Qu, *Huaqiao Daxue Xuebao, Ziran Kexueban*, 2000, **21**, 84.
21. D. Wen, Y. Liu and S. Qu, *Huaxue Yanjiu Yu Yingyong*, 1999, **11**, 419.
22. S.D. Dalosto, M.G. Ferreyra, R. Calvo, O.E. Piro and E.E. Castellano, *J. Inorg. Biochem.*, 1999, **73**, 151.
23. W. Hoffmüller, K. Polborn and W. Beck, *Z. Naturforsch.*, 1999, **54b**, 734.
24. R.F. de Farias and C. Airoldi, *J. Inorg. Biochem.*, 1999, **76**, 273.
25. G. Branica, N. Paulic, B. Grgas and D. Omanovic, *Chem. Speciation Bioavail.*, 1999, **11**, 125.
26. X. Yang, X. Zhang, M. Ji, S. Gao, F. Du and X. Liu, *Huaxue Yanjiu Yu Yingyong*, 2000, **12**, 318.
27. A.C. Rizzi, O.E. Piro, E.E. Castellano, O.R. Nascimento and C.D. Brondino, *Inorg. Chim. Acta*, 2000, **305**, 19.
28. E.J. Baran, C.C. Wagner, M.H. Torre, E. Kremer and P. Kogerler, *Acta Farm. Bonaerense*, 2000, **19**, 231.
29. R. Calvo, M.C.G. Passeggi, N.O. Moreno, G.E. Barberis, A.B. Chaves, B.C.M. Torres, L. Lezama and T. Rojo, *Phys. Rev. B. Condens. Matter Mater. Phys.*, 1999, **60**, 1197.
30. M.Q. Ehsan, M. Jebin, E. Ahmed and M.A. Quyser, *Indian J. Chem. Sect. A. Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 1999, **38A**, 826.
31. S.-L. Gao, Y.-D. Hou, J.-R. Liu, M. Ji and S.-P. Chen, *Huaxue Xuebao*, 2000, **58**, 65.
32. P.M. Zaitsev, V.V. Enger Aires, S.A. Kante, G.I. Dorofeeva, M.A. Zatikyan and N.U. Venskovskii, *Russ. J. Coord. Chem.*, 1999, **25**, 342.
33. H. Lavanant, E. Hecquet and Y. Hoppiliard, *Int. J. Mass. Spectrom.*, 1999, **185–187**,

11.
34. S.L. Gao, Y.D. Hou, Q. Wei, J.R. Liu and X.W. Yang, *Xibei Daxue Xuebao*, *Ziran Kexueban*, 1999, **29**, 517.
35. A.E. Gerbase, M. Martinelli, L.G. Fagundes, V. Stefani and S.J. Da Silva, *J. Coord. Chem.*, 1999, **47**, 451.
36. K. Majumder and S. Bhattacharya, *Polyhedron*, 1999, **18**, 3669.
37. T.A. Balakaeva, A.V. Churakov, M.G. Ezernitskaya, L.G. Kuzmina, B.V. Lokshin and I.A. Efimenko, *Russ. J. Coord. Chem.*, 1999, **25**, 579.
38. Z.P. Zheng and R.Y. Wang, *Comments on Inorg. Chem. Part A*, 2000, **22**, 1.
39. F. Ren, S.L. Gao, G.H. Chu and Q.Z. Shi, *Yingyong Huaxue*, 2000, **17**, 203.
40. Y. Du, H. Deng and S. Zhong, *Sichuan Daxue Xuebao*, *Ziran Kexueban*, 2000, **37**, 79.
41. Z. Li, C. Liu and K. Tan, *Zhongguo Yaowu Huaxue Zazhi*, 1999, **9**, 13.
42. S.R. de Andrade Leite, M.A. Counto dos Santos, C.R. Carubelli and A.M. Galindo Massabni, *Spectrochimica Acta*, *A*, 1999, **55**, 1185.
43. P. Drozdzewski and E. Kordon, *Spectrochim. Acta*, *A*, 2000, **56**, 2459.
44. P. Drozdzewski and E. Kordon, *Spectrochim. Acta*, *A*, 2000, **56**, 1299.
45. C.G. Zhang, C.Y. Duan, Q. Hu and D.Y. Yan, *J. Chem. Crystallogr.*, 1999, **29**, 1153.
46. F.B. Stocker, P. Fadden, S. Dreher and D. Britton, *Inorg. Chem.*, 1999, **38**, 3251.
47. R. Murugavel, V.V. Karambelkar, G. Anantharaman and M.G. Walawalkar, *Inorg. Chem.*, 2000, **39**, 1381.
48. Y. Inomata, K. Sasaki, H. Umehara and F.S. Howell, *Inorg. Chim. Acta*, 2001, **313**, 95.
49. D.W.M. Arrigan and L.L. Bihan, *Analyst* (*Cambridge*), 1999, **124**, 1645.
50. X. Dou, Y.M. Jung, Z.Q. Cao and Y. Ozaki, *Applied Spectr.*, 1999, **53**, 1440.
51. X. Zhao, Z. Gai, R.G. Zhao, W.S. Yang and T. Sakurai, *Surf. Sci.*, 1999, **424**, L347.
52. X. Zhao, *J. Am. Chem. Soc.*, 2000, **122**, 12584.
53. K. Imamura, T. Mimura, M. Okamoto, T. Sakiyama and K. Nakanishi, *J. Colloid Interface Sci.*, 2000, **229**, 237.
54. F. Huerta, E. Morallon, J.L. Vazquez and A. Aldaz, *J. Electroanal. Chem.*, 1999, **475**, 38.
55. G. Marques, J.L. Bourdelande and Valiente, *React. Funct. Polym.*, 1999, **41**, 77.
56. S. Stewart and P.M. Fredericks, *Spectrochimica Acta*, *A.*, 1999, **55**, 1641.
57. X. Yu, E. Gao, Z. Shu and H. Chen, *Huaxue Gongye Yu Gongcheng* (*Tianjin*), 1999, **16**, 249.
58. P. Sgarabotto, F. Bisceglie, G. Pelosi and L. Abdel-Rahman, *Polyhedron*, 1999, **18**, 2505.
59. M. Mizutani, S. Tomosue, H. Kinoshita, K. Jitsukawa, H. Masuda and H. Einaga, *Bull. Chem. Soc. Japan*, 1999, **72**, 981.
60. V.X. Jin and J.D. Ranford, *Inorg. Chim. Acta*, 2000, **304**, 38.
61. A.M. Nijasure, V.N. Joshi and A.D. Sawant, *J. Inorg. Biochem.*, 1999, **73**, 109.
62. C. Melián, C. Kremer, L. Suescun, A. Mombrú, R. Mariezcurrena and E. Kremer, *Inorg. Chim. Acta*, 2000, **306**, 70.
63. S. Pagliari, R. Corradini, G. Galaverna, S. Sforza, A. Dossena and R. Marchelli, *Tetrahedron Letters*, 2000, **41**, 3691.
64. P.R. Reddy and A.M. Reddy, *Proc. – Indian Acad. Sci., Chem. Sci.*, 2000, **112**, 593.
65. M. Mizutani, I. Kubo, K. Jitsukawa, H. Masuda and H. Einaga, *Inorg. Chem.*, 1999, **38**, 420.
66. J. Chin, S.S. Lee, K.J. Lee, S. Park and D.H. Kim, *Nature*, 1999, **401**, 254.
67. R. Barford, L. Bendahl, A. Hammershoi, D.K. Jensen, A.M. Sargeson and A.C.

Willis, *J. Chem. Soc. Dalton Trans.*, 1999, 449.
68. J. Cai, X. Hu, X. Feng, W. Shao, L. Ji and I. Bernal, *Eur. J. Inorg. Chem.*, 2000, 2199.
69. K. Kobayashi, H. Takahashi, M. Nishio, Y. Umezewa, K. Tsuboyama and S. Tsuboyama, *Anal. Sci.*, 2000, **16**, 1103.
70. K. Umakoshi, Y. Tsuruma, C.E. Oh, A. Takasawa, H. Yasukawa and Y. Sasaki, *Bull. Chem. Soc. Japan*, 1999, **72**, 433.
71. S. Kirsch, R. Jankowsky, P. Leibnitz, H. Spies and B. Johannsen, *J. Biol. Inorg. Chem.*, 1999, **4**, 48.
72. V. Noethig-Laslo and N. Paulic, *Polyhedron*, 1999, **18**, 1609.
73. B. Kaitner, N. Paulic, G. Pavlovicand J. Sabolovic, *Polyhedron*, 1999, **18**, 2301.
74. S. Zahn and J.W. Canary, *Science*, 2000, **288**, 1404.
75. M. Ashfaq, A. Majeed, A. Rauf, A.W.K. Khanzada, W.U. Shah and M.I. Ansari, *Bull. Chem. Soc. Japan*, 1999, **72**, 2073.
76. B. Macias, M.V. Villa, M. Martin-Simon and L.J. Rodriguez, *Transition Metal Chem.*, 1999, **24**, 533.
77. B. Macias, P. Malet, R. Paradinas, V. Rives and M.V. Villa, *Inorg. Chim. Acta*, 1999, **288**, 127.
78. D.S. Sagatys, C. Dahlgren, G. Smith, R.C. Bott and A.C. Willis, *Aust. J. Chem.*, 2000, **53**, 77.
79. K. Kawabe, M. Tadokoro, K. Hirotsu, N. Yanagihara and Y. Kojima, *Inorg. Chim. Acta*, 2000, **305**, 172.
80. N. Yanagihara, Y. Yumoto, K. Arai, K. Kawabe and Y. Kojima, *Polyhedron*, 2000, **19**, 1311.
81. K. Kawabe, M. Tadokoro, A. Ichimura, Y. Kojima, T. Takino and H. Sakurai, *J. Am. Chem. Soc.*, 1999, **121**, 7937.
82. P. Arranz-Mascarós, M.L. Godino-Salido, R. López-Garzón, M.D. Gutiérrez-Valero and J.M. Moreno, *Polyhedron*, 1999, **18**, 793.
83. J.M. Moreno, P. Arranz-Mascarós, R. López-Garzón, M.D. Gutiérrez-Valero, M.L. Godino-Salido and J. Cobo-Domingo, *Polyhedron*, 1999, **18**, 1635.
84. R. López-Garzón, P. Arranz-Mascarós, M.L. Godino-Salido, M.D. Gutiérrez-Valero, A. Pérez-Cadenas, J. Cobo-Domingo and J.M. Moreno, *Inorg. Chim. Acta*, 2000, **308**, 59.
85. P. Arranz-Mascarós, R. López-Garzón, M.D. Gutiérrez-Valero, M.L. Godino-Salido, and J.M. Moreno, *Inorg. Chim. Acta*, 2000, **304**, 137.
86. Á. García-Raso, J.J. Fiol, A. López-Zafra, A. Cabrero, I. Mata and E. Molins, *Polyhedron*, 1999, **18**, 871.
87. E. Colacio, M. Ghazi, R. Kivekäs and J.M. Moreno, *Inorg. Chem.*, 2000, **39**, 2882.
88. L.S. Long, S.P. Yang, Y.X. Tong, Z.W. Mao, X.M. Chen and L.N. Ji, *J. Chem. Soc. Dalton Trans.*, 1999, 1999.
89. A.S. Saghiyan, A.V. Geolchanyan, S.M. Djamgaryan, S.M. Vardapetyan, V.I. Tararov, N.A. Kuzmina, N.S. Ikonnikov, Yu.N. Belokon and M. North, *Russ. Chem. Bull.*, 2000, **49**, 1460.
90. A. García-Raso, J.J. Fiol, A. López-Zafra, I. Mata, E. Espinosa and E. Molins, *Polyhedron*, 2000, **19**, 673.
91. A. Erxleben and J. Hermann, *J. Chem. Soc. Dalton Trans.*, 2000, 569.
92. M.K. Gupta, H.L. Singh and A.K. Varshney, *ACGC Chem. Res. Commun.*, 1999, **9**, 20.
93. H.L. Singh, M. Sharma and A.K. Varshney, *Synth. React. Inorg. Met.-Org. Chem.*, 2000, **30**, 445.
94. H. Singh and A.K. Varshney, *ACGC Chem. Res. Commun.*, 1999, **9**, 9.

95. J.C. Pessoa, I. Cavaco, I. Correia, M.T. Duarte, R.D. Gillard, R.T. Henriques, F.J. Higes, C. Madeira and I. Tomaz, *Inorg. Chim. Acta*, 1999, **293**, 1.
96. K.M. Yao, W. Zhou, G. Lu and L.F. Shen, *Huaxue Xuebao*, 2000, **58**, 1275.
97. K. Haas, H. Nöth and W. Beck, *Z. Naturforsch.*, 1999, **54b**, 989.
98. A. Böhm, T. Hauck and W. Beck, *Z. Naturforsch.* 1999, **54b**, 1360.
99. B. Schreiner, R. Urban, A. Zografidis, K. Sünkel, K. Polborn and W. Beck, *Z. Naturforsch.*, 1999, **54b**, 970.
100. J. Cheng, K. Sunkel and W. Beck, *J. Prakt. Chem.* (*Weinheim, Ger.*), 1999, **341**, 792.
101. R. Urban, K. Polborn and W. Beck, *Z. Naturforsch.*, 1999, **54b**, 385.
102. O. Brosch, T. Weyhermüller and N. Metzler-Nolte, *Eur. J. Inorg. Chem.*, 2000, 323.
103. A. Hess, J. Sehnert, T. Weyhermüller and N. Metzler-Nolte, *Inorg. Chem.*, 2000, **39**, 5437.
104. R.S. Herrick, K.L. Houde, J.S. McDowell, L.P. Kiczek and G. Bonavia, *J. Organomet. Chem.*, 1999, **589**, 29.
105. H. Ait-Haddou, E. Bejan, J.C. Daran, G.G.A. Balavoine, F. Berruyer-Penaud, L. Bonazzola, H. Smaoui-Chaabouni and E. Amouyal, *J. Chem. Soc. Dalton Trans.*, 1999, 3095.
106. B. Geisser, A. Ponce and R. Alsfasser, *Inorg. Chem.*, 1999, **38**, 2030.
107. X. Zhang, W. Li, H. Jia and S. Weng, *Guangpuxue Yu Guangpu Fenxi*, 2000, **20**, 185.
108. S.L. De Wall, L.J. Barbour and G.W. Gokel, *J. Am. Chem. Soc.*, 1999, **121**, 8405.
109. V. Ryzhov and R.C. Dunbar, *J. Am. Chem. Soc.*, 1999, **121**, 2259.
110. S. Hoyau, K. Norrman, T.B. McMahon and G. Ohanessian, *J. Am. Chem. Soc.*, 1999, **121**, 8864.
111. T. Marino, N. Russo and M. Toscano, *J. Inorg. Biochem.*, 2000, **79**, 179.
112. R.A. Jockusch, W.D. Price and E.R. Williams, *J. Phys. Chem. A*, 1999, **103**, 9266.
113. B.A. Cerda and C. Wesdemiotis, *Analyst*, 2000, **125**, 657.
114. R.C. Dunbar, *J. Phys. Chem. A*, 2000, **104**, 8067.
115. T. Wyttenbach, M. Witt and M.T. Bowers, *J. Am. Chem. Soc.*, 2000, **122**, 3458.
116. R.M. Minyaev, A.G. Starikov and V.I. Minkin, *Mendeleev Commun.*, 2000, **10**, 43.
117. F. Rogalewicz, G. Ohanessian and N. Gresh, *J. Comput. Chem.*, 2000, **21**, 963.
118. B.A. Cerda and C. Wesdemiotis, *Int. J. Mass. Spectrom.*, 1999, **185–187**, 107.
119. J.M. Mercero, J.E. Fowler and J.M. Ugalde, *J. Phys. Chem. A*, 2000, **104**, 7053.
120. E.F. Strittmatter, A.S. Lemoff and E.R. Williams, *J. Phys. Chem. A*, 2000, **104**, 9793.
121. J. Sabolovicand K.R. Liedl, *Inorg. Chem.*, 1999, **38**, 2764.
122. S.J. Park, Y.K. Choi, S.S. Han and K.W. Lee, *Bull. Kor. Chem. Soc.*, 1999, **20**, 1475.
123. L.P. Loginova and O.S. Chernysheva, *J. Mol. Liq.*, 2000, **85**, 351.
124. A. Cuevas, I. Viera, M.H. Torre, E. Kremer, S.B. Etcheverry and E.J. Baran, *Afinidad*, 1999, **56**, 263.
125. C. Conato, A. Contino, G. Maccarrone, A. Magri, M. Remelli and G. Tabbi, *Thermochim. Acta*, 2000, **362**, 13.
126. T. Lezhava, N. Ananiashvili, M. Kikabidze and B. Tsanava, *Izv. Akad. Nauk Gruz., Ser. Khim.*, 2000, **26**, 26.
127. W.J. Puspita, M. Suzuki, D. Inoue, A. Odani, O. Yamauchi and T. Kiss, *Inorg. React. Mech.*, 1999, **2**, 137.
128. S.L. Gao, Y.D. Hou, M. Ji, S.P. Chen and Q.Z. Shi, *Thermochim. Acta*, 2000, **35**, 47.
129. F. Gharib, A.S. Sadjadi and F. Zourofi, *Rev. Inorg. Chem.*, 2000, **20**, 219.
130. M.F. Grigoreva and N.M. Kadyrkulova, *Vestn. S.-Peterb. Univ., Ser.* 4: *Fiz. Khim.*, 1999, 73.
131. G. Ye, C.X. Wang and S.S. Qu, *Wuhan Univ. J. Nat. Sci.*, 2000, **5**, 479.
132. H.B. Silber, N. Ghajari and V. Maraschin, *Mater. Sci. Forum*, 1999, **15–317**, 490.

133. B. Stypinski-Mis and G. Anderegg, *Anal. Chim. Acta*, 2000, **406**, 325.
134. L.F. Krylova and A.V. Golovin, *J. Struct. Chem.*, 2000, **41**, 254.
135. L.F. Krylova and A.V. Golovin, *J. Struct. Chem.*, 2000, **41**, 243.
136. E. Chruscinska, E. Garribba, G. Micera and A. Panzanelli, *J. Inorg. Biochem.*, 1999, **75**, 225.
137. F. Gharib and M. Mollaie, *J. Chem. Eng. Data*, 1999, **44**, 77.
138. H. Houkhani, M. Mollaie and F. Gharib, *Phys. Chem. Liquids*, 2000, **38**, 607.
139. M. Goanta and N. Foca, *A. Stiint. Univ.* '*Al. I. Cuza*' *Iasi*, *Chim.*, 1997, **5**, 87.
140. B. Grgas, S. Nikolic, Paulic, N. Paulic and N. Raos, *Croat. Chem. Acta*, 1999, **72**, 885.
141. B. Kurzak, A. Kamecka, K. Kurzak, J. Jezierska and P. Kafarski, *Polyhedron*, 2000, **19**, 2083.
142. E. Matczak-Jon, B. Kurzak, A. Kamecka, W. Sawka-Dobrowolska and P. Kafarski, *J. Chem. Soc. Dalton Trans.*, 1999, 3627.
143. L. Chruscinski, P. Mlynarz, K. Malinowska, J. Ochocki, B. Boduszek and H. Kozlowski, *Inorg. Chim. Acta*, 2000, **303**, 47.
144. T.R. Varga, R. Király, E. Brücher and V. Hietapelto, *Models in Chem.*, 1999, **136**, 431.
145. E. Farkas, H. Csóka, G. Bell, D.A. Brown, L.P. Cuffe, N.J. Fritzpatrick, W.K. Glass, W. Errington and T.J. Kemp, *J. Chem. Soc. Dalton Trans.*, 1999, 2789.
146. R.T. Yamaki, E.B. Paniago, S. Carvalho and I.S. Lula, *J. Chem. Soc. Dalton Trans.*, 1999, 4407.
147. E.G. Kontecka, G. Berthon, I.O. Fritsky, R. Wieczorek, Z. Latajka and H. Kozlowski, *J. Chem. Soc. Dalton Trans.*, 2000, 4201.
148. A. Dobosz, N.M. Dudarenko, I.O. Fritsky, T. Glowiak, A. Karaczyn, H. Kozlowski, T.Y. Silva and J.S. Kozlowska, *J. Chem. Soc. Dalton Trans.*, 1999, 743.
149. L.H. Abdel-Rahman, *Ann. Chim.*, 2000, **90**, 479.
150. T. Murakami, Z. Orihashi, Y. Kikuchi, S. Igarashi and Y. Yukawa, *Inorg. Chim. Acta*, 2000, **303**, 148.
151. M.M. Khalil and A.E. Attia, *J. Chem. Eng. Data*, 2000, **2**, 4870.
152. A. Zaidi and F. Khan, *J. Inst. Chem.*, 1998, **70**, 210.
153. A.A.A. Boraei, S.A. Ibrahim and A.H. Mohamed, *J. Chem. Eng. Data*, 1999, **44**, 907.
154. G.N. Mukherjee and S. Basu, *J. Indian Chem. Soc.*, 1999, **76**, 288.
155. M.S. Nair, G. Kalalakshmi and M.S. Pillai, *J. Indian Chem. Soc.*, 1999, **76**, 310.
156. P.L. Sahu and F. Khan, *Bull. Electrochem.*, 2000, **16**, 449.
157. G.U. Rani and D.D. Manwal, *Natl. Acad. Sci. Lett.*, (*India*), 1999, **22**, 12.
158. N.M. Guindi, *Egypt. J. Chem.*, 2000, **43**, 253.
159. N. Van Hoof, N.R. Russel, M. McNamara and R. Darcy, *J. Incl. Phenom. Macr. Chem.*, 2000, **36**, 179.
160. X.D. Zhang, Y. Liu, J.Y. Sun and Q.T. Liu, *Huaxue Xuebao*, 2000, **58**, 662.
161. E. Ross, R.J. Motekaitis and A.E. Martell, *Inorg. Chim. Acta*, 1999, **286**, 55.
162. T.F. Pauwels, W. Lippens, P.W. Smet, G.G. Herman and A.M. Goeminne, *Polyhedron*, 1999, **18**, 1029.
163. M.S. Nair, J.P. Anbarasi, P.T. Arasu and M.A. Neelakantan, *Indian J. Chem., Sect. A.: Inorg., Bio-inorg., Phys. Theor. Anal. Chem.*, 1999, **38A**, 166.
164. S. Vajhallya and F. Khan, *Bull. Chem. Soc. Japan*, 1999, **72**, 397.
165. A.I. El-Said, A.S.A. Zidan, M.S. El-Meligy, A.A.M. Aly and O.F. Mohammed, *Synth. React. Inor. Metal-org. Chem.*, 2000, **30**, 1373.
166. L.B. Saran, and M.N. Srivastava, *Rev. Roum. Chim.*, 1999, **44**, 125.
167. A.G. Devi and S. Satyanarayana, *J. Electrochem. Soc. India*, 1999, **48**, 275.

168. M.S. Nair, S.T. David and M. Anbu, *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 1999, **38A**, 823.
169. R.N. Patel, H.C. Pandey, K.B. Pandeya and G.N. Mukherjee, *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 1999, **38A**, 850.
170. E.A. Kasim and N.A. Abdalla, *Aswan Sci. Technol. Bull.*, 1997, **18**, 1.
171. R. Gupta, P.C. Vyas, M. Arora and R. Bapna, *Bull. Electrochem.*, 2000, **16**, 110.
172. A. Zaidi and F. Khan, *Bull. Electrochem.*, 2000, **16**, 119.
173. S. Çakir, E. Biçer and O. Çakir, *J. Inorg. Biochem.*, 1999, **77**, 249.
174. F. Khan and P.L. Sahu, *Ultra Sci. Phys. Sci.*, 2000, **12**, 106.
175. A. Zaidi and F. Khan, *Res. J. Chem. Environ.*, 1999, **3**, 47.
176. G.U. Rani and D.D. Manwal, *J. Electrochem. Soc. India*, 2000, **49**, 13.
177. N.M. Shuaib, H.M. Marafie, O. Al-Fulaij and M.S. El-Ezaby, *J. Chem. Eng. Data*, 1999, **44**, 1348.
178. Z.L. Chen, K. Uchiyama and T. Hobo, *Anal. Sci.*, 2000, **16**, 837.
179. T. Mizutani, K. Wada and S. Kitagawa, *J. Am. Chem. Soc.*, 1999, **121**, 11425.
180. X.B. Peng, J.W. Huang, T. Li and L.N. Ji, *Inorg. Chim. Acta*, 2000, **305**, 111.
181. S. Yagi, H. Sadachi, Y. Kashiwagi, T. Takagishi, T. Mizutani, S. Kitagawa and H. Ogoshi, *Chem. Lett.*, 2000, 1054.
182. T.J.M. de Bruin, A.T.M. Marcelis, H. Zuilhof and E.J.R. Sudholter, *Langmuir*, 2000, **16**, 8270.
183. M. Padmavathi and S. Satyanarayana, *Indian J. Chem., Sect A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 1999, **38A**, 295.
184. X. He, L. Long, F. Wu, X. Le, X. Chen, L. Ji and Z. Zhou, *Sci. China, Ser. B: Chem.*, 1999, **42**, 185.
185. E. Farkas, É.A. Enyedy, G. Micera and E. Garriba, *Polyhedron*, 2000, **19**, 1727.
186. Y. Yoshimura, *Bull. Chem. Soc. Japan*, 1999, 72, 1285.
187. Y. Yoshimura, *Bull. Chem. Soc. Japan*, 2000, **73**, 2739.
188. A. Kumar, P. Kumar and P. Ramamurthy, *Polyhedron*, 1999, **18**, 773.
189. S. Dubey and A. Pandey, *Oxid. Commun.*, 2000, **23**, 125.
190. N.N. Halligudi, S.M. Desai, S.K. Surekha and S.T. Nandibewoor, *Monatsh. Chem.*, 2000, **131**, 321.
191. M.R. Kembhavi, A.L. Harihar and S.T. Nandibewoor, *Oxid. Commun.*, 2000, **23**, 304.
192. A.L. Harihar, M.R. Kembhavi and S.T. Nandibewoor, *Monatsh. Chem.*, 2000, **131**, 739.
193. S. Dubey and A. Pandey, *Oxid. Commun.*, 2000, **23**, 451.
194. S. Dubey and A. Pandey, *Oxid. Commun.*, 2000, **23**, 443.
195. A.L. Harihar, M.R. Kembhavi and S.T. Nandibewoor, *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 2000, **39A**, 769.
196. H. Iloukhani and H. Bahrami, *Int. J. Chem. Kinet.*, 1999, **31**, 95.
197. H.S. Singh, B. Singh, A. Gupta and A.K. Singh, *Oxid. Commun.*, 1999, **22**, 146.
198. M. Choubey, S. Dubey and A. Pandey, *J. Inst. Chem. (India)*, 1999, **71**, 49.
199. A. Kumar, P.C. Mathur and K.S. Mathur, *J. Indian Counc. Chem.*, 1998, **15**, 75.
200. S. Chandraju, K.S. Rangappa and N.M. Gowda, *Int. J. Chem. Kinet.*, 1999, **31**, 525.
201. Z. Khan, *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 1999, **38A**, 361.
202. A.L. Harihar, R.G. Panari and S.T. Nadibewoor, *Oxid. Commun.*, 1999, **22**, 308.
203. R.B. Chougale, R.G. Panari and S.T. Nadibewoor, *Oxid. Commun.*, 1999, **22**, 298.
204. M. Choubey and A. Pandey, *Oxid. Commun.*, 1999, **22**, 293.
205. M.R. Kembhavi, A.L. Harihar and S.T. Nadibewoor, *J. Indian Chem. Soc.*, 1999, **76**,

79.
206. B.R. Sahu, V.R. Chourey, S. Pandey, L.V. Shastry and V.R. Shastry, *J. Indian Chem. Soc*., 1999, **76**, 131.
207. Z. Juan, G. Zijian, J.A. Parkinson, Y. Chen and P.J. Sadler, *Chem. Commun*., 1999, 1359.
208. Md. S.R. Kembhavi, R.G. Panari and S.T. Sharanappa, *Inorg. React. Mech.* (*Amsterdam*), 1999, **1**, 225.
209. J.F. Read, S.A. Bewick, C.R. Graves, J.M. MacPherson, J.C. Salah, A. Theriault and A.E.H. Wyand, *Inorg. Chim. Acta*, 2000, **303**, 244.
210. A.V. Kachur, C.J. Koch and J.E. Biaglow, *Free Radical Res*., 1999, **31**, 23.
211. A. Hanaki, T. Kinoshita and T. Ozawa, *Bull. Chem. Soc. Japan*, 2000, **73**, 73.
212. S.Y. Menshikov, A.V. Vurasko, L.S. Molochnikov, E.G. Kovalyova and A.A. Efendiev, *J. Mol. Catal. A*: *Chem*., 2000, **158**, 447.
213. T. Demappa and S. Ananda, *Asian J. Chem*., 1999, **11**, 376.
214. D. Berkeš, A. Kolarovic and F. Považanec, *Tetrahedron Letters*, 2000, **41**, 5257.
215. A.S. Demir, I.M. Akhmedov and O. Sesenoglu, *Turk. J. Chem*., 1999, **23**, 123.
216. K. Lemma, T. Shi and L.I. Elding, *Inorg. Chem*., 2000, **39**, 1728.
217. L. Chen, P.F. Lee, J.D. Ranford, J.J. Vittal and S.Y. Wong, *J. Chem. Soc. Dalton Trans*., 1999, 1209.
218. M. El-Khateeb, T.G. Appleton, L.R. Gahan, B.G. Charles, S.J. Berners-Price and A.M. Bolton, *J. Inorg. Biochem*., 1999, **77**, 13.
219. R. Karkalic and Z.D. Bugarcic, *Monatsh. Chem*., 2000, **131**, 819.
220. N. de Barrios, G. González, A. Grandas, M. Martinez and V. Moreno, *Inorg. React. Mechan*., 1999, **1**, 205.
221. A.K. Das, *Inorg. React. Mechan*., 2000, **1**, 309.
222. N.M. Guindy, Z.M. Abou-Gamra and M.F. Abdel-Messih, *J. Chim. Phys. Phys.-Chim. Biol*., 1999, **96**, 851.
223. H.C. Malhotra, S. Rattan and V. Singh, *Indian J. Chem*., *Sect. A*: *Inorg*., *Bio-inorg*., *Phys*., *Theor. Anal. Chem*., 1999, **38A**, 136.
224. A.R. Jr. Hill and L.E. Orgel, *Origins Life Evol. Biosphere*, 1999, **29**, 115.
225. G. Larrazabel, J. Parada and A. Decinti, *Bol. Soc. Chil. Quim*., 1999, **44**, 25.
226. O. Sijbren and J.B.F.N. Engberts, *J. Am. Chem. Soc*., 1999, **121**, 6798.
227. V.A. Soloshonok, C. Cai and V.J. Hruby, *Tetrahedron Lett*., 2000, **41**, 135.
228. T. Patonay, Cs. Hajdú, J. Jekő, A. Lévai, K. Micskei and C. Zucchi, *Tetrahedron Letters*, 1999, **40**, 1373.
229. J. Gyarmati, Cs. Hajdú, Z. Dinya, K. Micskei, C. Zucchi and Gy. Pályi, *J. Organomet. Chem*., 1999, **586**, 106.
230. U. Kazmaier and F.L. Zumpe, *Angew. Chem. Int. Ed*., 1999, **38**, 1468.
231. N. Niklas, O. Walter and R. Alsfasser, *Eur. J. Inorg. Chem*., 2000, 1723.
232. M. Berfeld, I. Kuzmenko, I. Weissbuch, H. Cohen, P.B. Howes, K. Kjaer, J. Als-Nielsen, L. Leiserowitz and M. Lahav, *J. Phys. Chem. B*, 1999, **103**, 6891.
233. A. Böhm and D. Seebach, *Helv. Chim. Acta*, 2000, **83**, 3262.
234. H. Tamiaki, N. Matsumoto, S. Unno, S. Shinoda and H. Tsukube, *Inorg. Chim. Acta*, 2000, **300–302**, 243.
235. S. Zhan, J.W. Canary, *Org. Lett*., 1999, **1**, 861.
236. J. Frelek, *Pol. J. Chem*., 1999, **73**, 229.
237. H. Tsukube, M. Wada, S. Shinoda and H. Tamiaki, *Kidorui*, 1999, **34**, 290.
238. H. Tsukube, M. Wada, S. Shinoda and H. Tamiaki, *Chem. Commun*., 1999, 1007.
239. Tao, D. Zhang, E. N. Nikolaev and R. G. Cooks, *J. Am. Chem. Soc*., 2000, **122**, 10598.

240. J.L. Seymur and F. Turecek, *J. Mass Spectrom.*, 2000, **35**, 566.
241. S.I. Aizawa, M. Okada, K.I. Kobayashi, S. Yamada and M. Nakamura, *Chem. Lett.*, 2000, 1412.
242. M. Schlauch, F.J. Volk, K.P. Fondekar, J. Wede and A.W. Frahm, *J. Chromatogr., A*, 2000, **897**, 145.
243. Z.L. Chen, J.M. Lin, K. Uchiyama and T. Hobo, *Anal. Sci.*, 2000, **16**, 131.
244. A. Dobashi and M. Hamada, *Electrophoresis*, 1999, **20**, 2761.
245. A.R. Mustafina, A.Y. Ziganshina, S.V. Fedorenko, E.K. Kazakova, V.V. Skripacheva and A.I. Konovalov, *Russ. J. Coord. Chem.*, 2000, **26**, 507.
246. C. Orvig and M. J. Abrams, eds., *Medicinal Inorganic Chemistry*, Special Issue of *Chem. Rev.*, 1999, **99**, 9.
247. N. Ohata, H. Masuda and O. Yamauchi, *Inorg. Chim. Acta*, 2000, **300–302**, 749.
248. R. Sankaranarayanan, A.C. Dock-Bregeon, B. Rees, M. Bovec, J. Caillet, P. Romby, C.S. Francklyn and D. Moras, *Nat. Struct. Biol.*, 2000, **7**, 461.
249. H. Kumita, N. Asai, T. Sakurai, K. Jitsukawa, T. Ozawa, H. Masuda and H. Einaga, *Inorg. Chem. Commun.*, 2000, **3**, 185.
250. P. Leverett, J. Petherick, P.A. Williams and R.S. Vagg, *J. Coord. Chem.*, 1999, **49**, 83.
251. H.M. Marques, O.Q. Munro, T. Munro, M. de Wet and P.R. Vashi, *Inorg. Chem.*, 1999, **38**, 2312.
252. J. Casanova, G. Alzuet, S. Ferrer, J. Latorre, J.A. Ramírez, J. Borrás, *Inorg. Chim. Acta*, 2000, **304**, 170.
253. D.I. Pattison, P.A. Lay and M.J. Davies, *Inorg. Chem.*, 2000, **39**, 2729.
254. C.T. Dillon, P.A. Lay, A.M. Bonin, M. Cholewa and G.J.F. Legge, *Chem. Res. Toxicol.*, 2000, **13**, 742.
255. O.E. Offiong, E. Nfor, A.A. Ayi and S. Martelli, *Trans. Met. Chem.*, 2000, **25**, 369.
256. S.B. Lee, S.C. Song, J.I. Jin and Y.S. Sohn, *Polym. J. (Tokyo)*, 1999, **31**, 1247.
257. C.E. Carraher Jr., F. Li, D. Siegmann-Louda, C. Butler, S. Harless and F. Pflueger, *Polym. Mater. Sci. Eng.*, 1999, **80**, 363.
258. D. Kong, Q. Chen, Y. Xie and X. Zhou, *Zhongguo Yaowu Huaxue Zazhi*, 2000, **10**, 13.
259. M. Sandow, B.L. May, C.J. Easton and S.F. Lincoln, *Aust. J. Chem.*, 2000, **53**, 149.
260. K. Jung, S. Ristori, G. Martini, *Spectrochimica Acta, A.*, 2000, **56**, 341.
261. C.J. Ziegler, K.E. Sandman, C.H. Liang and S.J. Lippard, *J. Biol. Inorg. Chem.*, 1999, **4**, 402.
262. E. Rodrígez-Fernández, E. García, M.R. Hermosa, A. Jiménez-Sánchez, M. Mar Sánchez, E. Monte and J.J. Criado, *J. Inorg. Biochem.*, 1999, **75**, 181.
263. H. Sakurai, Y. Kojima and H. Kanamori, *Jpn. Kokai Tokkyo Koho JP*, 2000, **44**, 584.
264. D. Shi, T.W. Hambley and H.C. Freeman, *J. Inorg. Biochem.*, 1999, **73**, 173.
265. M. Watabe, T. Kobayashi, T. Kawahashi, A. Hino, T. Watanabe, T. Mikami, T. Matsumoto and M. Suzuki, *J. Inorg. Biochem.*, 1999, **73**, 1.
266. M. Watabe, M. Kai, M. Hayashi, K. Kamiyama, H. Okada and T. Takayama, *J. Inorg. Biochem.*, 2000, **81**, 49.
267. W. Hoffmüller, R. Krämer, M. Maurus, K. Ppolborn and W. Beck, *Z. Naturforsch.*, 2000, **55b**, 855.
268. K. Haas, E.-M. Ehrenstorfer-Schäfers, K. Polborn and W. Beck, *Eur. J. Inorg. Chem.*, 1999, 465.
269. J. Costa Pessoa, I. Cavaco, I. Correia, D. Costa, R.T. Henriques and R.D. Gillard, *Inorg. Chim. Acta*, 2000, **305**, 7.
270. M. Biagioli, L. Strinna-Erre, G. Micera, A. Panzanelli and M. Zema, *Inorg. Chem.*

Comm., 1999, **2**, 214.
271. M. Bühl, *J. Inorg. Biochem*., 2000, **80**, 137.
272. A.J. Tasiopoulos, A.N. Troganis, A. Evangelou, C.P. Raptopoulou, A. Terzis, Y. Deligiannakis and T.A. Kabanos, *Chem. Eur. J*., 1999, **5**, 910.
273. A.J. Tasiopoulos, A.N. Troganis, Y. Deligiannakis, A. Evangelou, T.A. Kabanos, J. D. Woolins and A. Slawin, *J. Inorg. Biochem*., 2000, **79**, 159.
274. M. Nath, R. Yadav, G. Eng, T.-T. Nguyen and A. Kumar, *J. Organomet. Chem*., 1999, **577**, 1.
275. P. Tsiveriotis and N. Hadjiliadis, *J. Chem. Soc*., *Dalton Trans*., 1999, 459.
276. M.A. Girasolo, T. Pizzino, C. Mansueto, G. Valle and G.C. Stocco, *Appl. Organometal. Chem*., 2000, **14**, 197.
277. R.P. Bonomo, G. Impellizzieri, G. Pappalardo, E. Razzarelli and G. Tabbi, *Chem. Eur. J*., 2000, **6**, 4195.
278. K. Yamato, T. Inada, M. Doe, A. Ichimura, T. Takui, Y. Kojima, T. Kikunaga, S. Nakamura, N. Yanagihara, T. Onaka and S. Yano, *Bull. Chem. Soc. Jpn*., 2000, **73**, 903.
279. L. Santagostini, M. Gulotti, R. Pagliarin, E. Bianchi, L. Casella and E. Monzani, *Tetrahedron*: *Asymmetry*, 1999, **10**, 281.
280. C.A. Blasie and J.M. Berg, *Inorg. Chem*., 2000, **39**, 348.
281. X. Chen, M. Chu and D.P. Giedroc, *J. Biol. Inorg. Chem*., 2000, **5**, 93.
282. D. Fabris, Y. Hathout and C. Fenselau, *Inorg. Chem*., 1999, **38**, 1322.
283. M. Vargek, X. Zhao, Z. Lai, G.L. McLendon and T.G. Spiro, *Inorg. Chem*., 1999, **38**, 1372.
284. G. Veglia, F. Porcelli, T. DeSilva, A. Prantner and S.J. Opella, *J. Am. Chem. Soc*., 2000, **122**. 2389.
285. P. Faller, B. Ctortecka, W. Tröger, T. Butz, ISOLDE Collaboration and M. Vašak, *J. Biol. Inorg. Chem*., 2000, **5**, 393.
286. A. Munoz, F. Laib, D.H. Petering and C.F. Shaw III, *J. Biol. Inorg. Chem*., 1999, **4**, 495.
287. J. Mendieta, M.S. Diaz-Cruz, A. Monjonell, R. Tauler and M. Esteban, *Anal. Chim. Acta*, 1999, **390**, 15.
288. A.L. Nivorozhkin, B.M. Segal, K.B. Musgrave, S.A. Kates, B. Hedman, K.O. Hodgson and R.H. Holm, *Inorg. Chem*., 2000, **39**, 2306.
289. A. Tripathi, R.K. Syal and P.K. Bharadwaj, *Polyhedron*, 1999, **18**, 2229.
290. B.T. Farrer, C.P. McClure, J.E. Penner-Hahn and V.L. Pecoraro, *Inorg. Chem*., 2000, **39**, 5422.
291. M.J. Kelso, H.N. Hoang, T.G. Appleton and D.P. Fairlie, *J. Am. Chem. Soc*., 2000, **122**, 10488.
292. H. Wei, X. Luo, Y. Wu, Y. Yao, Z. Guo and L. Zhu, *J. Chem. Soc*., *Dalton Trans*., 2000, 4196.
293. E. Gaggeli, N. D'Amelio, N. Gaggeli and G. Valensin, *Eur. J. Inorg. Chem*., 2000, 1699.
294. O. Hénin, B. Barbier, F. Boillot and A. Brack, *Chem. Eur. J*., 1999, **5**, 218.
295. W. Bal, J. Wojcik, M. Maciejczyk, P. Grochowski and K.S. Kasprzak, *Chem. Res. Toxicol*., 2000, **13**, 823.
296. W. Bal, J. Wojcik, M. Maciejczyk and K.S. Kasprzak, *J. Biomol. Structr. Dyn*., 1999, **16**, 1370.
297. X.-F. Qi, B.S. Zhorov and V.S. Ananthanarayanan, *J. Pept. Sci*., 2000, **6**, 57.
298. W.Y. Feng, S. Gronert and C.B. Lebrilla, *J. Am. Chem. Soc*., 1999, **121**, 1365.
299. N. Niklas, O. Walter and R. Alsfasser, *Eur. J. Inorg. Chem*., 2000, 1723.

300. J.M. Rowland, M.M. Olmstead and P.K. Mascharak, *Inorg. Chem.*, 2000, **39**, 5326.
301. A.K. Patra, M. Ray and R. Mukherjee, *Inorg. Chem.*, 2000, **39**, 652.
302. I.O. Fritsky, *J. Chem. Soc., Dalton Trans.*, 1999, 825.
303. P. Comba, S.P. Gavrish, Y.D. Lampeka, P. Lightfoot and A. Peters, *J. Chem. Soc., Dalton Trans.*, 1999, 4099.
304. B. König, T. Fricke, K. Gloe and C. Chartroux, *Eur. J. Inorg. Chem.*, 1999, 1557.
305. G. Greiner, L. Seyfarth, W. Popitz, R. Witter, U. Sternberg and S. Reissmann, *Lett. Pept. Sci.*, 2000, **7**, 133.
306. A. Bonamarti Corradi, G. Lusvardi, L. Menabue, M. Saladini and P. Sgarabotto, *Polyhedron*, 1999, **18**, 1975.
307. M. Borsari, L. Menabue and M. Saladini, *Polyhedron*, 1999, **18**, 1983.
308. M. Saladini, D. Iacopino and L. Menabue, *J. Inorg. Biochem.*, 2000, **78**, 355.
309. J.J. Becker, P.S. White and M.R. Gagné, *Inorg. Chem.*, 1999, **38**, 798.
310. M.A. Ashraf, K. Jones and S. Handa, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1617.
311. W. Guangbin, *Spectrosc. Lett.*, 1999, **32**, 679.
312. T.E. Lehmann, M.L. Serrano and L. Que Jr., *Biochemistry*, 2000, **39**, 3886.
313. Y. Hara, L. Shen, A. Tsubouchi, M. Akiyama and K. Umemoto, *Inorg. Chem.*, 2000, **39**, 5074.
314. T. Szabó-Planka, A. Rockenbauer and L. Korecz, *Magn. Res. Chem.*, 1999, **37**, 484.
315. T. Szabó-Planka, N. Nagy, A. Rockenbauer and L. Korecz, *Polyhedron*, 2000, **19**, 2049.
316. S. Bruni, F. Cariati, P.G. Daniele and E. Prenesti, *Spectrochim. Acta*, 2000, **56A**, 815.
317. E. Prenesti, P.G. Daniele, M. Prencipe and G. Ostacoli, *Polyhedron*, 1999, **18**, 3233.
318. S. Rabouan, J. Delage, W. Durand, P. Prognon and D. Barthes, *Talanta*, 2000, **51**, 787.
319. Y. Zhang, S. Akilesh and D.E. Wilcox, *Inorg. Chem.*, 2000, **39**, 3057.
320. C.G. Ágoston, T. Kowalik Jankowska and I. Sóvágó, *J. Chem. Soc., Dalton Trans.*, 1999, 3295.
321. A. Hanaki, T. Kawashima, T. Konishi, T. Takano, D. Mabuchi, A. Odani and O. Yamauchi, *J. Inorg. Biochem.*, 1999, **77**, 147.
322. P. Surdy, P. Rubini, N. Buzás, B. Henry, L. Pellerito and T. Gajda, *Inorg. Chem.*, 1999, **38**, 346.
323. A. Jancsó, B. Henry, P. Rubini, G. Vankó and T. Gajda, *J. Chem. Soc., Dalton Trans.*, 2000, 1941.
324. N. Buzás, L. Nagy, H. Jankovics, R. Kramer, E. Kuzmann, A. Vértes and K. Burger, *J. Radioanal. Nucl. Chem.*, 1999, **241**, 313.
325. A.A. Al-Najjar, M.R. Shehata, M.M.A. Mohamed and M.M. Shoukry, *Main Group Metal Chem.*, 1999, **22**, 253.
326. W.J. Puspita, A. Odani and O. Yamauchi, *J. Inorg. Biochem.*, 1999, **73**, 203.
327. P. Mlynarz, W. Bal, T. Kowalik-Jankowska, M. Stasiak, M.T. Leplawy and H. Kozlowski, *J. Chem. Soc., Dalton Trans.*, 1999, 109.
328. P. Mlynarz, N. Gaggeli, J. Panek, M. Stasiak, G. Valensin, T. Kowalik-Jankowska, M.L. Leplawy, Z. Latajka and H. Kozlowski, *J. Chem. Soc., Dalton Trans.*, 2000, 1033.
329. P. Mlynarz, T. Kowalik-Jankowska, M. Stasiak, M.T. Leplawy and H. Kozlowski, *J. Chem. Soc., Dalton Trans.*, 1999, 3673.
330. T. Kowalik-Jankowska, W. Leśniak, J. Brasuń, M. Stasiak and M.T. Leplawy, *Polyhedron*, 2000, **19**, 241.
331. K. Várnagy, J. Szabó, I. Sóvágó, G. Malandrinos, N. Hadjiliadis, D. Sanna and G.

Micera, *J. Chem. Soc., Dalton Trans.*, 2000, 467.
332. M.A. Zoroddu, T. Kowalik-Jankowska, H. Kozlowski, H. Molinari, K. Salnikow, L. Broday and M. Costa, *Biochim. Biophys. Acta*, 2000, **1475**, 163.
333. T. Kowalik-Jankowska, M. Jasionowski and L. Łankiewicz, *J. Inorg. Biochem.*, 1999, **76**, 63.
334. M. Casolaro, M. Chelli, M. Ginanneschi, F. Laschi, M. Muniz-Miranda, A.M. Papini and G. Sbrana, *Spectrochim. Acta*, 1999, **55A**, 1675.
335. T. Kowalik-Jankowska, M. Ruta-Dolejsz, K. Wiśniewska, L. Łankiewicz and H. Kozlowski, *J. Chem. Soc., Dalton Trans.*, 2000, 4511.
336. K. Várnagy, I, Sóvágó, H. Süli-Vargha, D. Sanna and G. Micera, *J. Inorg. Biochem.*, 2000, **81**, 35.
337. U. Herr, W. Spahl, G. Trojandt, W. Steglich, F. Thaler and R. van Eldik, *Bioorg. Med. Chem.*, 1999, **7**, 699.
338. M. Remelli, C. Conato, A. Agarossi, F. Pulidori, P. Mlynarz and H. Kozlowski, *Polyhedron*, 2000, **19**, 2409.
339. M.G. Bugarin, and M. Filella, *J. Inorg. Biochem.*, 1999, **73**, 17.
340. W. Bal, V. Karantza, E.N. Moudrianakis and K.S. Kasprzak, *Archives Biochem. Biophys.*, 1999, **364**, 161.
341. G. McLendon, H. Hull, K. Larkin and W. Chang, *J. Biol. Inorg. Chem.*, 1999, **4**, 171.
342. B. Quintanilla-Vega, D.J. Hoover, W. Bal, E.K. Silbergeld, M.P. Waalkes and L.D. Anderson, *Chem. Res. Toxicol.*, 2000, **13**, 594.
343. B. Quintanilla-Vega, D.J. Hoover, W. Bal, E.K. Silbergeld, M.P. Waalkes and L.D. Anderson, *Am. J. Industr. Med.*, 2000, **38**, 324.
344. J.C. Payne, M.A. ter Horst and H.A. Godwin, *J. Am. Chem. Soc.*, 1999, **121**, 6850.
345. C.G. Ágoston, K. Várnagy, A. Bényei, D. Sanna, G. Micera and I. Sóvágó, *Polyhedron*, 2000, **19**, 1849.
346. L. Grøndahl, N. Sokolenko, G. Abbenante, D.P. Fairlie, G.R. Hanson and L.R. Gahan, *J. Chem. Soc., Dalton Trans.*, 1999, 1227.
347. E. Lodyga-Chruscinska, G. Micera, D. Sanna, J. Olczak, J. Zabrocki, H. Kozlowski and L. Chruscinski, *J. Inorg. Biochem.*, 1999, **76**, 1.
348. E. Lodyga-Chruscinska, E. Brzezinska-Blaszczyk, G. Micera, D. Sanna, H. Kozlowski J. Olczak, J. Zabrocki and A.K. Olejnik, *J. Inorg. Biochem.*, 2000, **78**, 283.
349. N.A. Dobrynina, M.E. Akateva and L.S. Nikolaeva, *Zh. Fiz. Khim.*, 2000, **74**, 942.
350. J. Swiatek-Kozlowska, J. Brasun, L. Chruscinski, E. Chruscinska, M. Makowski and H. Kozlowski, *New. J. Chem.*, 2000, **24**. 893.
351. J. Brasun, P. Ciapetti, H. Kozlowski, S. Oldziej, M. Taddei, D. Valensin, G. Valensin and N. Gaggelli, *J. Chem. Soc., Dalton Trans.*, 2000, 2639.
352. E.C. O'Brien, E. Farkas, A. Rockenbauer and K.B. Nolan, *J. Inorg. Biochem.*, 1999, **77**, 135.
353. M.S. Nair and G. Subbalakshmi, *Indian J. Chem.*, 2000, **39A**, 468.
354. M.S. Nair and G. Subbalakshmi, *J. Indian Chem. Soc.*, 2000, **77**, 442.
355. R.N. Patel and K.B. Pandeya, *Indian J. Biochem. Biophys.*, 2000, **37**, 334.
356. R.N. Patel, P.R. Gokhale and K.B. Pandeya, *J. Indian Chem. Soc.*, 1999, **76**, 475.
357. M.S. Nair and G. Subbalakshmi, *J. Indian Chem. Soc.* 2000, **77**, 26.
358. P.R. Reddy and K.F. Nightingale, *Indian J. Chem.*, 2000, **39A**, 1024.
359. P.R. Reddy and K.F. Nightingale, *Indian J. Chem.*, 2000, **39A**, 1157.
360. X. Li, Y. Liang and L. Jin, *J. Mol. Structr.*, 2000, **525**, 261.
361. M. Borsari, L. Menabue and M. Saladini, *Polyhedron*, 1999, **18**, 1983.
362. J.C. Buschbaum and J.M. Berg, *Inorg. Chim. Acta*, 2000, **297**, 217.
363. S. Dey, M. Ray and P. Banerjee, *Inorg. React. Mechanisms*, 2000, **2**, 267.

364. J. Bujdák and B.M. Rode, *J. Mol. Catal.*, 1999, **144A**, 129.
365. J. Bujdák and B.M. Rode, *Origins of Life Evol. Biosphere*, 1999, **29**, 451.
366. T.N. Parac, G.M. Ullmann and N.M. Kostić, *J. Am. Chem. Soc.*, 1999, **121**, 3127.
367. M.I. Djuran and S.U. Milinković, *Polyhedron*, 1999, **18**, 3611.
368. M.I. Djuran and S.U. Milinković, *Polyhedron*, 2000, **19**, 959.
369. M.I. Djuran and S.U. Milinković, *Monatsh Chem.*, 1999, **130**, 613.
370. S. Zhou, Z. Yu and L. Zhu, *Wuji Huaxue Xuebao*, 1999, **15**, 497.
371. N.V. Kaminskaya, T.W. Johnson and N.M. Kostić, *J. Am. Chem. Soc.*, 1999, **121**, 8663.
372. N.V. Kaminskaya, G.M. Ullmann, D.B. Fulton and N.M. Kostić, *Inorg. Chem.*, 2000, **39**, 5004.
373. X. Luo, W. He, Y. Zhang, Z. Guo and L. Zhu, *Chin. J. Chem.*, 2000, **18**, 855.
374. F. Qiao, J. Hu, H. Zhu, X. Luo, L. Zhu and D. Zhu, *Polyhedron*, 1999, **18**. 1629.
375. L.M. Berreau, M.M. Makowska-Grzyska and A.M. Arif, *Inorg. Chem.*, 2000, **39**, 4390.
376. X.S. Than, Y. Fujii, T. Sato, Y. Nakano and M. Yashiro, *Chem. Commun.*, 1999, 881.
377. M.T. Bordignon Luiz, B. Szpoganicz, M. Rizzoto, M.G. Basallote and A.E. Marthell, *Inorg. Chim. Acta*, 1999, **287**, 134.
378. T. Takarada, M. Yashiro and M. Komiyama, *Chem. Eur. J.*, 2000, **6**, 3906.
379. S. Nishino, M. Kunita, Y. Kani, S. Ohba, H. Matsushima, T. Tokii and Y. Nishida, *Inorg. Chem. Commun.*, 2000, **3**, 145.
380. S.I. Murahashi, A. Mitani and K. Kitao, *Tetrahedron Letters*, 2000, **41**, 10245.
381. M. Khossravi and R.T. Borchardt, *Pharm. Res.*, 2000, **17**, 851.
382. W. Bal, L. Liang, J. Lukszo, S.-H. Lee, M. Dizdaroglu and K.S. Kasprzak, *Chem. Res. Toxicol.*, 2000, **13**, 616.
383. N. Kato, M. Nakamura and T. Uchiyama, *J. Inorg. Biochem.*, 1999, **75**, 117.
384. K. Lemma, J. Berglund, N. Farrell and L.I. Elding, *J. Biol. Inorg. Chem.*, 2000, **5**, 300.
385. T. Won, Y. Lee and D. Kim, *Anal. Sci. Technol.*, 1999, **12**, 460.
386. M. Hahn, D. Wolters, W.S. Sheldrick, F.B. Hulsbergen and J. Reedijk, *J. Biol. Inorg. Chem.*, 1999, **4**, 412.
387. J-M. Teuben and J. Reedijk, *J. Biol. Inorg. Chem.*, 2000, **5**, 463.
388. D. Wolters and W.S. Sheldrick, *J. Chem. Soc., Dalton Trans.*, 1999, 1121.
389. Z. Nagy, I. Fábián and I. Sóvágó, *J. Inorg. Biochem.*, 2000, **79**, 129.
390. M.I. Djuran and S.U. Milinković;, *Aust. J. Chem.*, 2000, **53**, 645.
391. P. del Soccorro Murdoch, N.A. Kratochwil, J.A. Parkinson, M. Patriarca and P.J. Sadler, *Angew. Chem. Int. Ed.*, 1999, **38**, 2949.
392. M. Beltrán, G.B. Onoa, E. Pedroso, V. Moreno and A. Grandas, *J. Biol. Inorg. Chem.*, 1999, **4**, 701.
393. R. Pogni, M.C. Baratto, E. Busi and R. Basosi, *J. Inorg. Biochem.*, 1999, **73**, 157.
394. J. Ueda, A. Hanaki, K. Hatano and T. Nakajima, *Chem. Pharm. Bull.*, 2000, **48**, 908.
395. E.M. Shoukry, M.M. Shoukry, A.E. Mahgoub and H.M. Galal, *Ann. Chim.*, 2000, **90**, 593.
396. R. Liang, S. Senturker, X. Shi, W. Bal, M. Dizdaroglu and K.S. Kasprzak, *Carcinogenesis*, 1999, **20**, 893.
397. A. Corazza, F. Vianello, L. Zennaro, N. Guorova, M.L. Di Paolo, L. Signor, O. Marin, A. Rigo and M. Scarpa, *J. Peptide Sci.*, 1999, **54**, 491.
398. M.J. Alcón, M. Iglesias, F. Sánchez and I. Viani, *J. Organomet. Chem.*, 2000, **601**, 284.
399. A. Kufelnicki and M. Swiatek, *Polish J. Chem.*, 1999, **73**, 579.

400. P. Saweczko and H.B. Kraatz, *Coord. Chem. Rev.*, 1999, **190–192**, 185.
401. W. Bauer, K. Polborn and W. Beck, *J. Organomet. Chem.*, 1999, **579**, 269.
402. O. Brosch, T. Weyhermüller and N. Metzler-Nolte, *Inorg. Chem.*, 1999, **38**, 5308.
403. A. Hess, O. Brosch, T. Weyhermüller and N. Metzler-Nolte, *J. Organomet. Chem.*, 1999, **589**, 75.
404. J. Wonnemann, M. Oberhoff, G. Erker, R. Fröchlich and K. Bergander, *Eur. J. Inorg. Chem.*, 1999, 1111.
405. A. Böhm, K. Polborn and W. Beck, *Z. Naturforsch.*, 1999, **54b**, 300.
406. A. Böhm and W. Beck, *J. Organomet. Chem.*, 1999, **588**, 247.
407. W. Hoffmüller, R. Krämer, M. Maurus, K. Polborn and W. Beck, *Z. Naturforsch.*, 2000, **55b**, 855.
408. M.A. Stalteri, S. Bansal, R. Hider and S.J. Mather, *Bioconjugate Chem.*, 1999, **10**, 130.
409. X. Zhang, Z.-F. Su, J.R. Ballinger, A.M. Rauth, A. Pollak and J.R. Thornback, *Bioconjugate Chem.*, 2000, **11**, 401.
410. A. Pollak, D.G. Roe, C.M. Pollak, L.F.L. Lu and J.R. Thornback, *J. Am. Chem. Soc.*, 1999, **121**, 11593.
411. H.P. Vanbilloen, B.J. Cleynhens and A.M. Verbruggen, *Nucl. Med. Biol.*, 2000, **27**, 207.
412. G.H. Van Domselaar, S.M. Okarvi, M. Fanta, M.R. Suresh and D.S. Wishart, *J. Labelled Compd. Radiopharm.*, 2000, **43**, 1193.
413. V. Polyakov, V. Sharma, J.L. Dahlheimer, C.M. Pica, G.D. Luker and D. Piwnica-Worms, *Bioconjugate Chem.*, 2000, **11**, 762.
414. S. Liu, D.S. Edwards, A.R. Harris, S.J. Heminway and J.A. Barrett, *Inorg. Chem.*, 1999, **38**, 1326.
415. B. Nock, T. Maina, F. Tisato, C.P. Raptopoulou, A. Terzis and E. Chiotellis, *Inorg. Chem.*, 2000, **39**, 5197.
416. C. Decristoforo and S.J. Mather, *Bioconjugate Chem.*, 1999, **10**, 431.
417. J.W. Babich, W.G. Coco, S. Barrow, A.J. Fischman, F.J. Femia and J. Zubieta, *Inorg. Chim. Acta*, 2000, **309**, 123.
418. A. Heppeler, S. Froidevaux, H.R. Mäcke, E. Jermann, M. Béhé, P. Powell and M. Hennig, *Chem. Eur. J.*, 1999, **5**, 1974.
419. A. Mushtaq, S. Pervez and I. Haider, *Radiochim. Acta*, 2000, **88**, 495.
420. J.S. Lewis, M.R. Lewis, A. Srinivasan, M.A. Scmidt, J. Wang and C.J. Anderson, *J. Med. Chem.*, 1999, **42**, 1341.
421. A. Kurihara and W.M. Pardridge, *Bioconjugate Chem.*, 2000, **11**, 380.
422. M.S. Robillard, A.Rob P.M. Valentijn, N.J. Meeuwenoord, G.A. van der Marel, J.H. van Boom and J. Reedijk, *Angew, Chem. Int. Ed.*, 2000, **39**, 3096.
423. K. Tanaka, K. Shigemori and M. Shionoya, *Chem. Commun.*, 1999, 2475.
424. P. Rossi, F. Felluga, P. Tecilla, F. Formaggio, M. Crisma, C. Toniolo and P. Scrimin, *J. Am. Chem. Soc.*, 1999, **121**, 6948.
425. M. Conti, G. Falini and B. Samori, *Angew. Chem. Int. Ed.*, 2000, **39**, 215.
426. Y. Chen, R. Pasquinelli, M. Ataai, R.R. Koepsel, T.W. Stringfield and R.E. Shepherd, *J. Inorg. Biochem.*, 1999, **76**, 211.
427. Y. Chen, R. Pasquinelli, M. Ataai, R.R. Koepsel, R.A. Kortes and R.E. Shepherd, *Inorg. Chem.*, 2000, **39**, 1180.
428. F. Dallavalle, G. Folesani, G. Pelosi, M.B. Ferrari, G. Galaverna, R. Corradini and R. Marchelli, *J. Coord. Chem.*, 2000, **51**, 135.
429. S. Aizawa, M. Okada, K. Kobayashi, S. Yamada and M. Nakamura, *Chem. Letters*, 2000, 1412.

430. L. Mao and K. Yamamoto, *Electroanalysis*, 2000, **12**, 577.
431. I. Hamachi, Y. Yamada, T. Matsugi and S. Shinkai, *Chem Eur. J*. 1999, **5**, 1503.
432. Y. Shi and S. Sharma, *Bioorg*., *Med. Chem. Letters*, 1999, **9**, 1469.
433. S. Ranganathan and N. Tamilarasu, *Inorg. Chem*., 1999, **38**, 1019.
434. M.P. McCurdie and L.A. Belfiore, *J. Polymer Sci. B*, 1999, **37**, 301.
435. L.A. Belfiore and M.P. McCurdie, *Polym. Eng. Sci*., 2000, **40**, 738.
436. A. Hess and N. Metzler-Nolte, *Chem. Commun*., 1999, 885.
437. J.C. Verheijen, G.A. van der Marel, J.H. van Boom and N. Metzler-Nolte, *Bioconjugate Chem*., 2000, **11**, 741.
438. J.G. Muller, L.A. Kayser, S.J. Paikoff, V. Duarte, N. Tang, R.J. Perez, S.E. Rokita and C.J. Burrows, *Coord. Chem. Rev*., 1999, **185–186**, 761.
439. R.P. Houser, M.P. Fitzsimons and J.K. Barton, *Inorg. Chem*., 1999, **38**, 1368.
440. D.L. Huffman and K.S. Suslick, *Inorg. Chem*., 2000, **39**, 5418.
441. T. Ueno, N. Nishikawa, S. Moriyama, S. Adachi, K. Lee, T. Okamura, N. Ueyama and A. Nakamura, *Inorg. Chem*., 1999, **38**, 1199.
442. C.L. Torres-Martinez, L. Nguyen, R. Kho, W. Bae, K. Bozhilov, V. Klimov and R.K. Mehra, *Nanotechnology*, 1999, **10**, 340.
443. J.M. Slocik and R.E. Shepherd, *Inorg. Chim. Acta*, 2000, **311**, 80.

6
Proteins

BY GRAHAM C. BARRETT

1 Introduction*

This chapter concentrates on new growing points in protein science, as reported in the literature for 2000. These significant advances have opened up as a result of the ongoing collection of results for proteins in newly-investigated organisms, and through revisiting well-studied systems, by applying new laboratory methodology and new knowledge gained in cognate areas of science.

Emphasis is placed on papers describing results with interpretations that are based on molecular structure. The papers selected are representative of the year's literature, in terms of content and use of imagery. The descriptive biochemistry of proteins is not covered.

'Increased understanding of the roles of proteins in cellular processes' is the most obvious phrase by which the new knowledge can be summarised in a few words. Although most of the themes represented in current protein research would be familiar to chemists and biochemists who were working in this field several years ago, the fine detail can be appreciated only against the current context in which new results are set.

Since progress in this area leads to opportunities for clinical intervention using designed proteins and low molecular weight mimics, the considerable investment for expanding protein research in the pharmaceutical industry, clearly demonstrated in the current literature, is easily understandable.

2 Structure of This Chapter

This chapter is the successor to the sequence of annual reviews, 'Trends in Protein Research', published in recent volumes of this Specialist Periodical Report to cover the literature 1990–1999. These predecessor chapters have used a selective coverage of the literature of protein science, an approach that is needed to fit a review of this vast field into a manageable space. The same general policy has been adopted again, but the approach adopted in previous chapters,

* Most of the protein structures reproduced in this chapter are in colour in the original sources. Authors have usually chosen to use colour to help the reader to appreciate the discussion of their work, and the on-line version of this chapter reproduces the colour versions. This can be found at http://www.rsc.org/ebooks/CONTENTS/AA002033/AA033006figures.pdf.

Amino Acids, Peptides and Proteins, Volume 33

in which topics for the coverage of proteins in a particular volume were chosen so as to cover gaps in preceding volumes (Vol. 32, p. 357), has been discarded in favour of attempting to develop a more enduring framework.

2.1 Cross-referencing in This Chapter. – A particular research paper in protein science can often be classified under more than one subsection of this chapter and cross-referencing has been provided to cope with this, to help the reader to appreciate the relationship of recent papers to broader themes.

3 Textbooks and Monographs

Protein topics have been covered in several textbooks and monographs published during the year. Most of these are mentioned in the relevant sections of this chapter (Sections 4.1, 4.2, 4.4, 5, 7.1, and 8.1), while recent titles that are of general relevance to protein chemistry and biochemistry are collected here. These are: *Protein Kinase Protocols*,[1] *Protein Structure Prediction*,[2] *Protein Architecture*,[3] *Handbook of Metalloproteins*,[4] *Protein Purification*,[5] *Proteins, Peptides and Amino Acids Sourcebook*,[6] *Proteins*: *Biochemistry and Biotechnology*,[7] and another *Handbook on Metalloproteins*.[8]

Some significant textbooks that were published in earlier years, but not cited in preceding volumes of this Specialist Periodical Report, are listed here: *Structure and Mechanism in Protein Science*, by A. Fersht, *Introduction to Protein Structure*, second edition, by C. Branden and J. Tooze and *Comprehensive Biological Catalysis*, ed. M. L. Sinnott,[9] are well established; *Proteins*, ed. R. H. Angeletti and *Methods for the Investigation of Amino Acid and Protein Metabolism*, ed. A. E. El-Khoury,[10] are also noteworthy.

3.1 Literature Searching in Protein Science. – Current awareness sources specifically providing titles of papers in protein science are mostly located in the biological sciences sections of the library, even though the main thrust of these papers is based on protein chemistry. Up to date information (titles of around 15 000 papers per year) is listed in *Current Advances in Protein Biochemistry* (issues appear monthly; papers are grouped into conventional sub-divisions).[11] The long-running series *Advances in Protein Chemistry* has continued to appear due to the efforts of various editors and publishers.[12]

The internet is now often the first resort as a source of information on proteins and the large number of websites competing to include the string 'protein' leads to confusing duplication in their titles – *e.g.* proteinscience.org and proteinscience.com insist that they have nothing to do with each other (the first of these is linked to the Protein Society journal, *Protein Science*, and the second is a site centred around the thriving proteomics field).

3.2 Protein Nomenclature. – The pronouncements of Committees of IUPAC and of IUBMB (and of its predecessor, IUB) on protein and polypeptide nomenclature are conveniently accessible at http://www.chem.qmw.ac.uk/iupac/. Rec-

ommendations that are collected at this site this year are unchanged from last year. This is a testament to the skills and efforts expended 20 years and more ago, on nomenclature matters.

4 Structure Determination of Proteins

4.1 Proteomics and Genomics. – A thorough coverage of progress and techniques in this rapidly developing area of research is becoming increasingly available in textbooks and monographs. Textbooks include *Introduction to Proteomics*,[13] *Genomics and Proteomics*: *Functional and Computational Aspects*,[14] *Application of Proteomics*,[15] and *Bioinformatics*: *A Practical Guide to the Analysis of Genes and Proteins*[16] (see also ref. 19).

In recent years, the number of novel proteins identified through genomic and proteomic projects has dramatically increased, with a concomitant need for more rapid determination of their tertiary structures. Although genomic data currently available for higher organisms appear to contain considerable levels of errors[17] leading to faulty protein sequences, modifications to the current successful approach are not likely. The assistance of computational techniques in protein structure assignments has spawned the new subject of 'functional genomics', which will ease the task of assignments of protein function.[18]

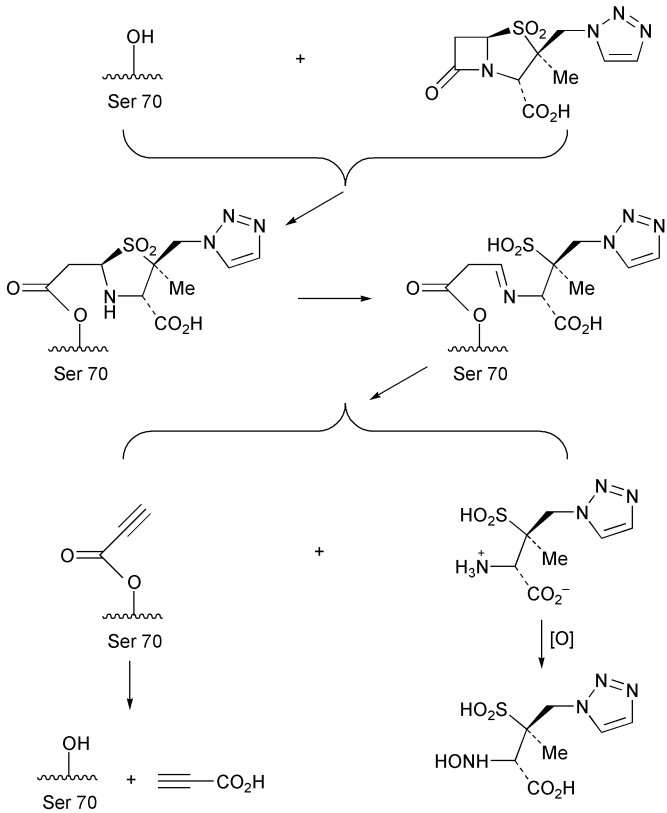
The objectives of proteomics are firmly based in applications of structural and analytical techniques that have reached sophisticated levels. Large scale analysis of proteins is an essential starting point for the assessment of protein–protein interactions, with mass spectrometry[19] and results from the yeast two-hybrid system being commonly used for characterization.[20]

4.2 Mass Spectrometry. – Recent textbooks cover mass spectrometry of proteins and peptides,[21] and protein sequencing and identification using tandem mass spectrometry.[22]

The power of current mass spectrometric methods is also clearly revealed in studies of proteins and other complex molecules, in areas outside the field of proteomics. It has been used for qualitative and quantitative characterization of catalysed ester hydrolysis of *p*-nitrophenyl α-glucopyranoside by α-glucosidase and of resorufin by bovine lipoprotein lipase. This study was a prelude to the study of proteolysis of viral protein capsids to provide information about capsid dynamics and the stabilizing force of viral protein/RNA interactions.[23]

The monitoring of hydrogen exchange in entire proteins by ^{1}H–^{2}H-exchange by matrix-assisted laser desorption ionisation, time of flight (MALDI-TOF) mass spectrometry, makes it possible to check the folding state of a protein and to characterize the conformational consequences of site-directed mutagenesis.[24]

The mechanism of breakdown of tazobactam by Class A β-lactamases PCI and TEM-1 follows from studies by electrospray MS, and turns out to be very similar to the ways in which clavulanic acid is inactivated (Scheme 1; see also ref. 167).[25] Other studies of lactamases (refs. 164, 165) and lactamase inhibitors (ref. 166) are discussed later in this chapter.



Scheme 1 *Acylation of residue 70 of β-lactamase by tazobactam, and a route (one of several alternatives) leading to breakdown of the antibiotic to propiolic acid and a hydroxyamino acid*
(After Yang *et al.*[25])

4.3 Three-dimensional Structures of Proteins Determined Using Physical Methods in Combination with Structural Derivatization. – Protein fold identification can be determined by a combination of crosslinking using derivatization by a disuccinimidyl ester, followed by mass spectrometry. Visualization of the three-dimensional structures of proteins has traditionally been achieved by X-ray crystallography and by NMR. These techniques produce high resolution atomic data but require relatively large amounts (10 to 100 mg) of pure analyte in a particular solution or crystalline state. Even if these conditions are met, it can take months or even years to generate a molecular structure by following these methodologies.

To develop an alternative approach, cross-linking technology has been re-examined in the light of newer analytical protocols for the separation and identification of complex peptide mixtures. Previous investigators have shown that cross-linking experiments can provide low resolution interatomic distance information and, given enough distance information, it is possible to solve the

tertiary structure of a macromolecule. To validate a model of human erythropoietin, a rapid method for identifying cross-linked residues by mass spectrometry has been developed, involving disuccinimidyl ester cross-linking and Edman sequencing and mass spectrometry of proteolytically digested, cross-linked proteins.

This use of chemical cross-linking and TOF-mass spectrometry to identify Lys-Lys cross-links, from which the fold of a protein can be identified, has been illustrated for basic fibroblast growth factor (FGF)-2, a protein for which both NMR-derived and crystallographic structures are available. A major aim of this work was to aid the construction of a homology model.[26]

The kinesin sub-family UNC104 has been characterized using a combination of limited proteolysis, mass spectrometry and physicochemical analysis. This classical approach to structure determination shows that the protein contains an FHA domain that locates the site of protein–protein interactions regulated by phosphorylation.[27]

4.4 Nuclear Magnetic Resonance Spectroscopy (NMR). – For a textbook see *Protein Dynamics Using NMR Relaxation*.[28]

^{15}N- and ^{1}H-NMR of samples under variable pressure provides information on the environment of histidine residues in a novel protein from *Staphylococcus carnosus*.[29]

Current routine NMR techniques of great sophistication, more powerful than those of even a few years ago, can give three-dimensional information from spectra obtained directly on proteins. As an example of current methodology (see also refs. 57, 79, 97, 103), sequence 1–156 of the α-subunit of spectrin has been analysed by NMR;[30] a standard graphic depiction of the results is included in this paper, but the results are easily appreciated in words: residues 1–20 = random; 21–45 = α-helix; 46–52 = random; 53–81 = α-helix; 82–87 = random; 88–118 = α-helix; 119–122 = random; 123–153 = α-helix.

Solid-state NMR is a technique that can give a useful volume of information for insoluble proteins and has been applied to silk characterisation. DOQSY analysis of Eri silkworm fibres (*Samia cynthia ricini*), very similar to spider dragline protein, has been reported, providing details of secondary structure at localities in the protein, in terms of torsion angles ϕ and φ of the backbone groupings.[31]

Accurate and rapid docking of protein–protein complexes has been visualised on the basis of intermolecular nuclear Overhauser enhancement (nOe) data and dipolar couplings by rigid body minimization. For proteins whose structures are available in the unbound state, and provided that no significant backbone conformational changes occur upon complexation (which can readily be assessed by analysis of dipolar couplings measured on the complex), accurate and rapid docking of the two proteins can be pictured. This turns out to be a simple and rapid method for solving the three-dimensional structures of protein–protein complexes in solution on the basis of experimental NMR restraints that provide the requisite translational data (derived from intermolecular nOe) and orientational information (*i.e.* backbone ^{1}H–^{15}N dipolar couplings and inter-

molecular nOes). The method, which has been demonstrated for the 40 kDa complex of an enzyme with the histidine phosphocarrier protein, involves the application of rigid body minimization using a target function comprising only three terms, namely experimental nOe-derived intermolecular interproton distance and dipolar coupling restraints, and a simple intermolecular van der Waals repulsion potential.

This approach promises to reduce the amount of time and effort required to solve the structures of protein–protein complexes by NMR, and to extend the capabilities of NMR to larger protein–protein complexes, possibly up to molecular masses of 100 kDa and beyond.[32]

4.5 X-Ray Crystallographic Studies. – The technique has become almost routine with the availability of improved instrumentation and needs no special coverage here in its own right. Discussions of interpretations of protein behaviour that depend on crystal structures (refs. 53, 58, 65, 87, 102, 105, 106, 108, 113, 114, 116, 117, 123, 128, 138, 148, 157–159, 190, 191) are scattered throughout this chapter.

5 Folding and Conformational Studies

This topic is developing in a number of ways, notably in the acquisition of novel insights into the effects of atom–atom interactions on local conformational and configurational features (increasingly based on *cis- versus trans*-peptide bond-containing sequences). These interactions can involve hydrogen bonding, but ligand–domain interactions and metal ion–domain interactions continue to be the targets of many research studies.

Recent textbooks include *Protein Folding, Evolution and Design*,[33] *From Protein Folding to New Enzymes*,[34] *Protein Structure, Stability and Folding*,[35] and *Mechanisms of Protein Folding*.[36]

5.1 Background to Protein Folding Studies. – A fundamental question in molecular biology asks how proteins fold into domains that can serve as assembly modules for building up large macromolecular structures. There are continuing attempts to set protein folding into a rational process that can be described in mechanical detail, the inevitable 'best structure' being reached iteratively. Much scope exists for unintended diversions of the folding process, including accelerations and decelerations along the pathway, these being determined by factors such as temperature and make-up of the medium in which the protein is being studied.

As an example of current work in this field, the biogenesis of pili on the surface of Gram-negative bacteria is a topic of current interest, in view of the role of these external features in locomotion of the organism. This biogenesis requires the orchestration of a complex process that includes protein synthesis, folding *via* small chaperones, secretion and assembly. The hypothesis that pilus subunit folding and biogenesis proceed *via* donor strand complementation and donor

strand exchange mechanisms, is supported by a study that shows that the steric information necessary for pilus subunit folding is not contained in only one polypeptide sequence. The missing information is transiently donated by a separate molecule, a strand of a small chaperone, to allow folding. In this work, the missing information for folding was supplied by adding a 13-amino acid residue peptide to the C-terminal end of a pilus subunit polypeptide. This resulted in the production of a protein that no longer required a chaperone to enable it to fold.[37]

5.2 Mechanics of Protein Folding. – The fundamental physics of the pathway leading a protein to its native conformation, starting either from its totally random version or from the point at which the protein is synthesized at the ribosome, may be simpler and more robust than previously thought.[38] A new view of protein folding, the conformation flow of ensembles rather than stepwise conformational changes within specific structures,[39] has permeated into some studies.

The reverse process can be important in the cell context, and the roles of topology and energetics on the unfolding of proteins through external forces and temperature have been considered.[40]

Structural studies of psychrophilic (cold-adapted) proteins are capable of giving information on subtle aspects of the behaviour of their mesophilic counterparts. Helices of 'cold trypsins' lack four of the hydrogen bonds and two of the salt bridges of their normal versions (Figures 1–3) and therefore have poorer van der Waals packing interactions within the body of the molecule.[41] The figures show structures for bovine trypsin and salmon trypsin, with structural details established by homology modelling techniques, backed up by comparison with X-ray structures established in earlier years.

Reports of well-studied proteins appear in the current literature as well as papers on fresh new facts. Conformational aspects of ribonuclease A are regularly revisited (Figure 4; see also ref. 199), and a new assessment of the contribution of the disulfide bonds of this enzyme to its conformational stability and catalytic activity (and other properties) has been reported.[42]

Phosphate ions accelerate the oxidative folding of reduced bovine ribonuclease A by dithiothreitol (the native enzyme contains four disulfide bridges) through stabilization of intermediates.[43] The role of simple inorganic anions in stabilizing the three-dimensional structure of a soluble protein has been well-known for many years, but more detailed information on the micro-environments set up by the ions is now being acquired, as illustrated in this study.

Bovine mitochondrial F_1-ATPase has been subjected to residue-exchange studies to reveal several helix-stabilizing centres in the native enzyme (Figure 5).[44]

Many papers on molecular mechanics assessments of small peptides, as models for protein fragments, are published each year. These are not covered thoroughly in this chapter, but newly-introduced generally applicable techniques are noted. Leap-dynamics, a molecular simulation scheme for sampling protein conformational space,[45] offers an improvement over the classical molecular dynamics approach since it can detect conformational flexibility correspond-

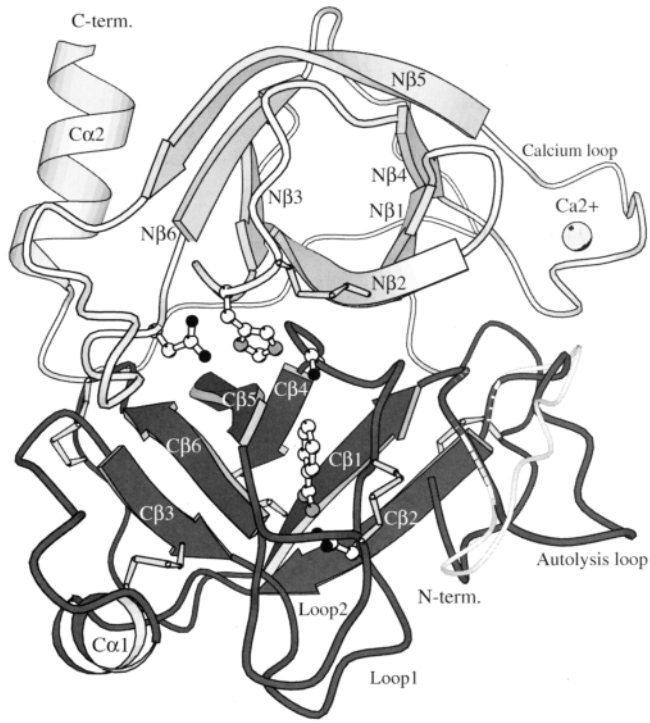


Figure 1 *Global structure for anionic salmon trypsin overlaid with the autolysis loop of bovine trypsin (residues 141–155 in light grey)*
(Reproduced with permission from Leiros *et al.*,[41] *Eur. J. Biochem.*, Blackwell Science)

ing to slow motions on the timescale that is familiar from NMR data. The 'alanine dipeptide' *N*-Ac-L-Ala-NHMe was used as a test-bed for the method, and its applicability to well-known proteins was demonstrated.

5.3 Misfolding and Unfolding of Proteins (see also Section 7.7). – The tendency to form amyloid fibrils under certain conditions is a generic property of polypeptide chains. New examples are being added to the well-known sequences that promote aggregation when dissolved in common solvents. Thus, dilution with water of a solution in 50% aqueous acetonitrile, of sequence 1–22 of cold shock protein CspB from *Bacillus subtilis*, causes precipitation.[46] Misfolding of hen egg lysozyme occurs in concentrated ethanolic solution, from which amyloid protofilaments soon start to deposit.[47]

Thermal unfolding of G-actin and β-actin (Figure 6; see also ref. 143) has been assessed, since it results in loss of ability to inhibit DNA-ase-I.[48]

A *Naja naja* basic protein fragment has been sequenced, and a molecular model constructed based on sequence homology with other snake venom neurotoxins.[49]

Structures of the intradiskal loops and the amino-terminal stretch of the G-protein receptor rhodopsin indicate that the loops are independently stabil-

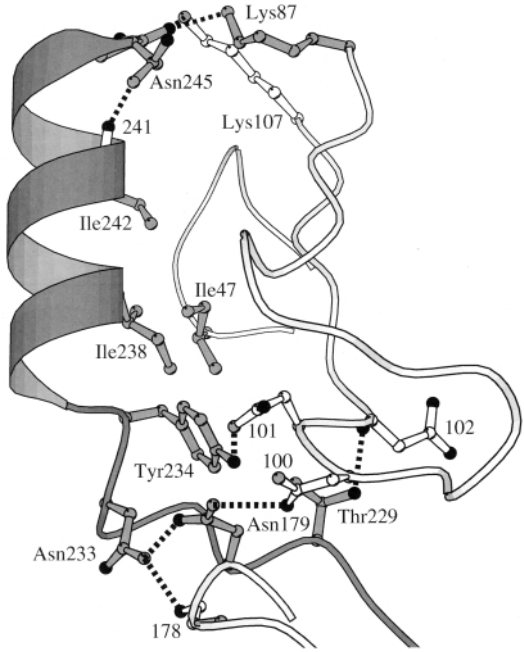


Figure 2 *Interactions for the C-terminal α-helix found in CST and mammalian trypsin that are not possible in the psychrophilic trypsins. Featured are: ion-pair formation from Lys87 to the C-terminal carboxy group; intrahelical hydrogen bond Asn245 to Nδ2 of residue 241; three isoleucine residues forming the hydrophobic core I (Ile47, Ile238, Ile242); three hydrogen bonds connecting the start of the helix to the rest of the molecule (233–178; 233–179, 234–101). The Asp100–Asn179 and Thr229–Asp102 hydrogen bonds are also featured*
(Reproduced with permission from Leiros *et al.*,[41] *Eur. J. Biochem.*, Blackwell Science)

ized and that helices extend outwards from the highly hydrophobic transmembrane region. The helix–turn–helix motif may be a recurring structural element of membrane proteins.[50]

Duck δ2-crystallin tetramer undergoes reversible dissociation and denaturation in aqueous guanidine hydrochloride (Figure 7; see also refs. 71–73).[51]

6 Protein–Metal Complexes

For textbooks relevant to this section, see refs. 4, 8.

6.1 Effects of Metal Complexation on Protein Structure. – Reversible complex formation by divalent Mn and Ca ions with the lectin concanavalin A (see also ref. 90) determines carbohydrate binding through inducing large conformational changes governed by Ala207-Asp208 peptide bond isomerization.[52] This study succeeds in identifying regions where deformations follow the binding of metal

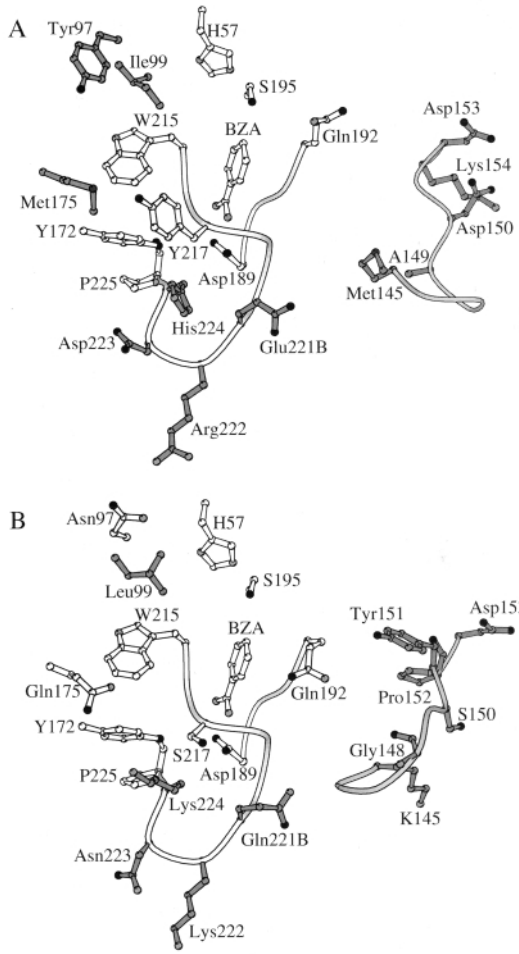


Figure 3 *(A) Structural features around the active site of psychrophilic cod trypsin (features shared with cold trypsin are shown in grey). (B) Structural features around the active site of bovine trypsin (features shared with mammalian trypsin shown in grey)*
(Reproduced with permission from Leiros *et al.*,[41] *Eur. J. Biochem.*, Blackwell Science)

ions (Figures 8–12). In Figure 9 the metal-binding loop is shown, together with the conserved metal ligand protein residues (Glu8, Asp10, Asn14, Asp19 and His24), metal ligand water molecules, the Ala207-Asp208 dipeptide in ball-and-stick model and second shell metal ligand residue Ser34. Water molecules are identified within this structure, bonded to the metal ligands. This is not unexpected, but the discovery that one water molecule is excluded from the S1 site is notable and is deduced after establishing the conformation for the side chain of Asp19, which leaves no space for a water molecule (Figure 10).

The factors promoting alkali-metal ion binding by proteins are little under-

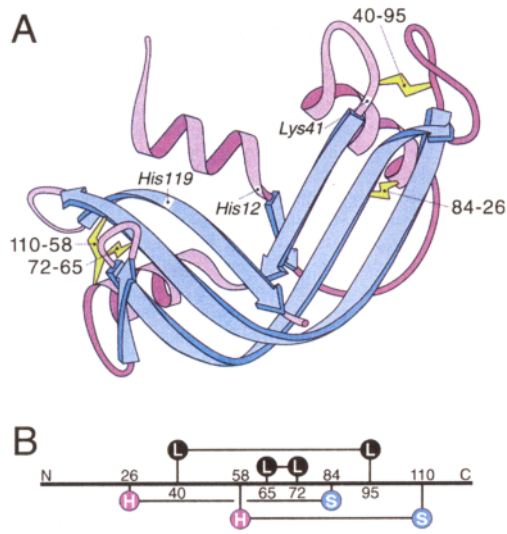


Figure 4 *(A) Ribbon diagram for ribonuclease A with inscriptions referring to the location of the disulfide bonds and active-site residues. The solvent-accessible surface area (0.52 nm² = 100%) of the cystine side chains in the crystalline protein are Cys2–Cys84, 0 nm²; Cys58–Cys110, 0 nm²; Cys26–Cys84, 0.02 nm²; Cys40–Cys95, 0.06 nm²; Cys65–Cys72, 0 nm². (B) The connectivity of the half-cystine residues is indicated by H = α-helix, L = surface loop and S = β-sheet*
(Reproduced with permission from Klink *et al.*,[42] *Eur. J. Biochem.*, Blackwell Science)

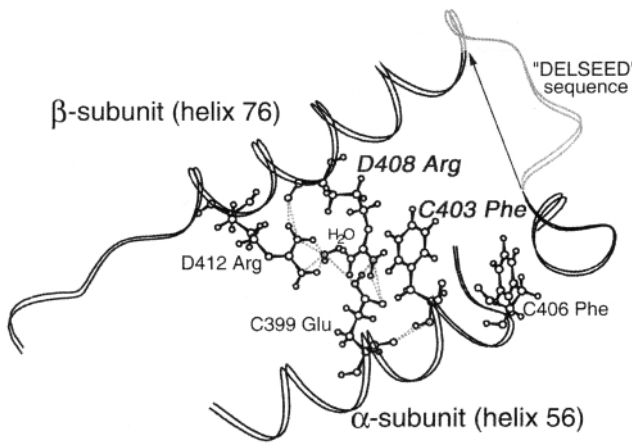


Figure 5 *Residues considered likely to stabilize helix 76 in the β-subunit and helix 56 in the α-subunit of the ADP-binding domain of mitochondrial F_1-ATPase*
(Reproduced with permission from Clark-Walker *et al.*[44] and Elsevier Science)

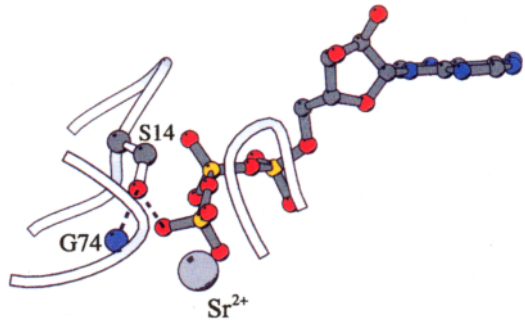


Figure 6 *Nucleotide-binding site of β-actin (see also Figure 53). The OH group of Ser14 in the phosphate-binding loop of actin subdomain I is within hydrogen-bonding distance of the γ-phosphate of ATP and the backbone amide group of Gly74*
(Reproduced with permission from Schuler *et al.*,[48] *FEBS Lett.*, Blackwell Science)

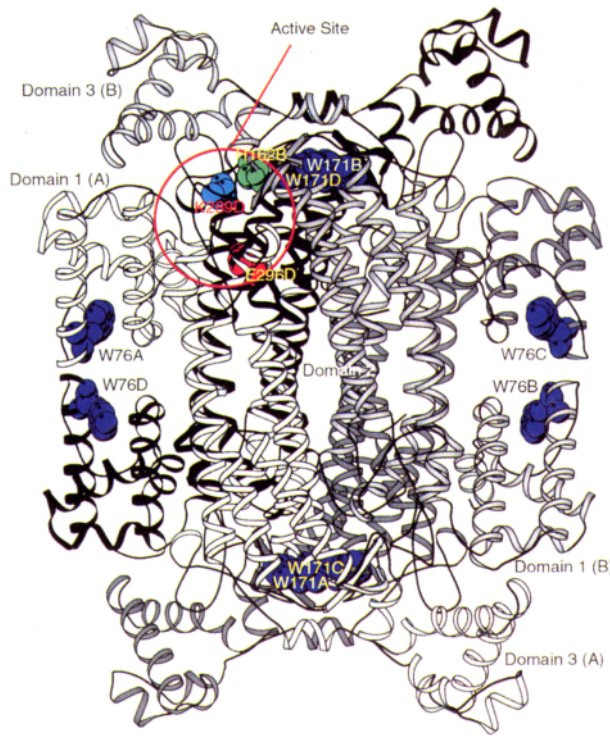


Figure 7 *The tetrameric structure of δ-crystallin. The active site is indicated. Subunits A (white) and B (grey) are shown in front. There is close interaction between the three domains of subunits A and B*
(Reproduced with permission from Lee and Chang,[51] *Eur. J. Biochem.*, Blackwell Science)

Figure 8 *Three-dimensional structure of the concanavanin A monomer. The S1 site is the smaller white sphere and the S2 site is the larger black sphere. The dark β-strands are those whose conformations are affected by the binding of calcium. The* cis*-peptide bond isomerization that ensues does not involve proline residues or locking of concanavanin A. The locations of the residues Thr11, Ser96 and Glu102 and of the* cis*-peptide bond on each of these dark β-strands are highlighted*
(Reproduced with permission from Bouckaert *et al.*[52])

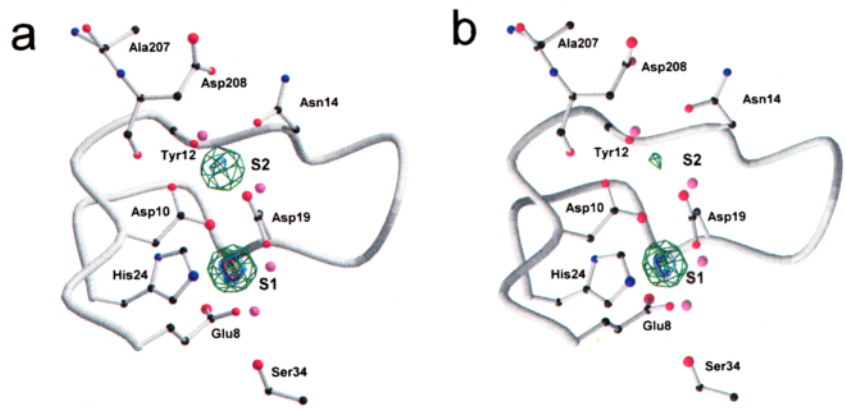


Figure 9 *Anomalous-difference Fourier electron density in the S1 and S2 sites of (a) LMnMn concanavanin A (*i.e. *the protein + ligand + 2Mn system compared with (b) LMnCa concanavanin A (*i.e. *the protein + ligand + Mn + Ca system). The metal binding loop is shown, together with the conserved metal ligand protein residues (Glu8, Asp10, Asn14, Asp19 and His24), metal ligand water molecules as purple spheres, the Ala207–Asp208 dipeptide as a ball-and-stick structure and the second shell metal ligand residue Ser34. The displayed electron density is brought to the same level in the S1 site that contains manganese in both structures to enable comparison of the electron density in the S2 sites (levels of electron density are shown in green, light blue, dark blue, and red)*
(Reproduced with permission from Bouckaert *et al.*[52])

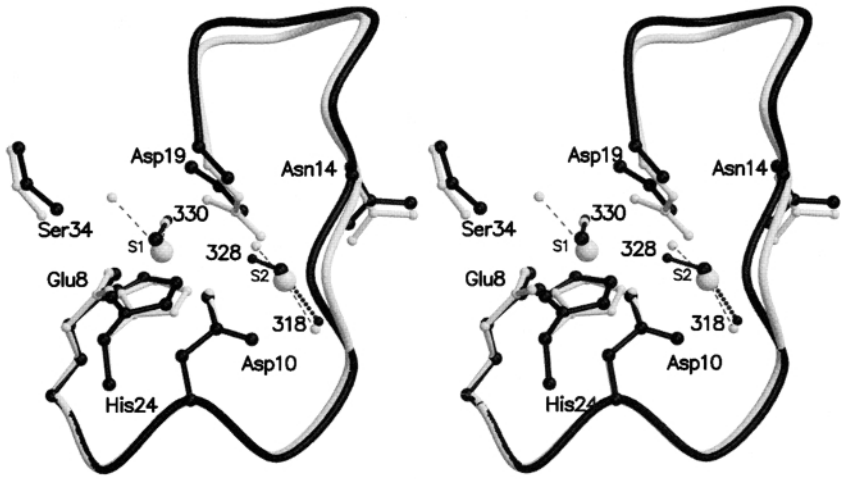


Figure 10 *Stereo picture of the superposition of the binuclear metal binding site of L concanavanin A (black) and LMnCa concanavanin A (*i.e. *the protein + ligand + Mn + Ca system; white). Hydrogen bonds are indicated by thin, grey (LMnCa concanavanin A) or thick, dark (L concanavanin A) dashed lines. The manganese (S1 site) and calcium (S2 site) of LMnCa concanavanin A are replaced by water molecules 303 and 311, respectively, in L concanavanin A. These make hydrogen bonds to the usual metal ligand water molecules, except for one water molecule that is excluded from the S1 site because of the different conformation of the side chain of Asp19*
(Reproduced with permission from Bouckaert *et al.*[52])

stood, even though the role of such ions in protein behaviour, particularly the acceleration of enzyme catalytic steps, is well known. The reconsideration of structure of tryptophanase that has led to identification of an alkali metal cation bound to an aromatic side chain, instead of an assumed water molecule (Figure 13), is part of a study of synthetic alkali-metal ion receptors.[53] The ability of K^+ to interact with an aromatic ring was assessed in this study by preparing a family of synthetic receptors that incorporate the aromatic side chains of phenylalanine, tyrosine and tryptophan. These receptors are constructed around a diaza-18-crown-6 scaffold, which serves as the primary binding site for an alkali metal cation.

From this reconsideration of a local region of an enzyme structure, the general attitude towards assignments of electron density for protein crystal structures is given a healthy jolt. What would have been assigned by experienced crystallographers as a water molecule bound to an aromatic grouping, by interpretation of electron density features, is now shown to be equally well assigned as an alkali metal cation for which π-interaction with the aromatic moiety is consistent with information obtained for model systems.

6.2 Membrane Proteins (see also Section 9.1). – The observation that membrane proteins generally pack more tightly than soluble proteins has important

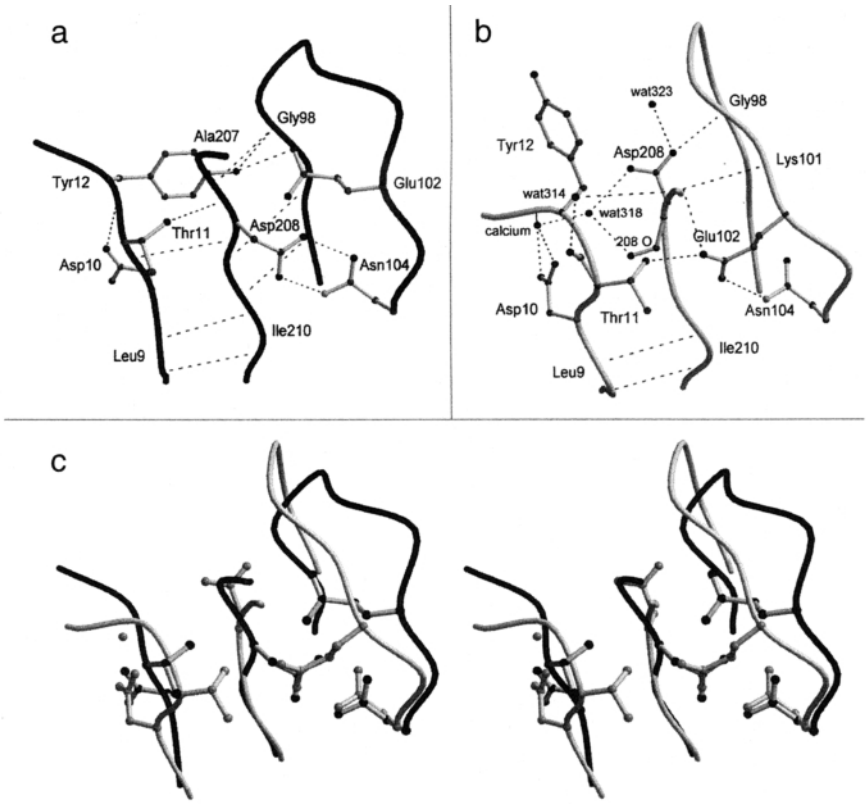


Figure 11 *Structural differences between the locked and unlocked forms of metal-free concanavanin A, emphasizing the hydrogen-bonding network involving strands Ile4–Asp10, Pro206–Asn216, Trp88–Thr97 and Thr103–Ser117. (a) Unlocked metal-free concanavanin A. (b) Locked metal-free concanavanin A. (c) Stereo view of the superposition of unlocked and locked metal-free concanavanin A. In (a) and (b), the hydrogen bond interactions are shown as dashed lines, and residues are labelled. The backbone is black for unlocked concanavanin A and white for locked concanavanin A. wat = water*
(Reproduced with permission from Bouckaert *et al.*[52])

implications for their stability and function. The stability of membrane-embedded and soluble proteins is comparable, despite the fact that membrane proteins do not rely on the hydrophobic effect to drive protein folding.

Detailed packing interactions clearly contribute a significant energetic component to membrane protein stability and are likely to play a leading role in guiding helix association in membrane protein folding. Packing analysis of the helical portions of seven integral membrane proteins and thirty-seven soluble proteins show that the helices in membrane proteins have higher packing values (0.431) than in soluble proteins (0.405). The highest packing values in integral membrane proteins originate from small hydrophobic (glycine and alanine) and small hydroxyalkyl amino acids (serine and threonine), whereas in soluble proteins large hydrophobic and aromatic residues have the highest packing values.[54]

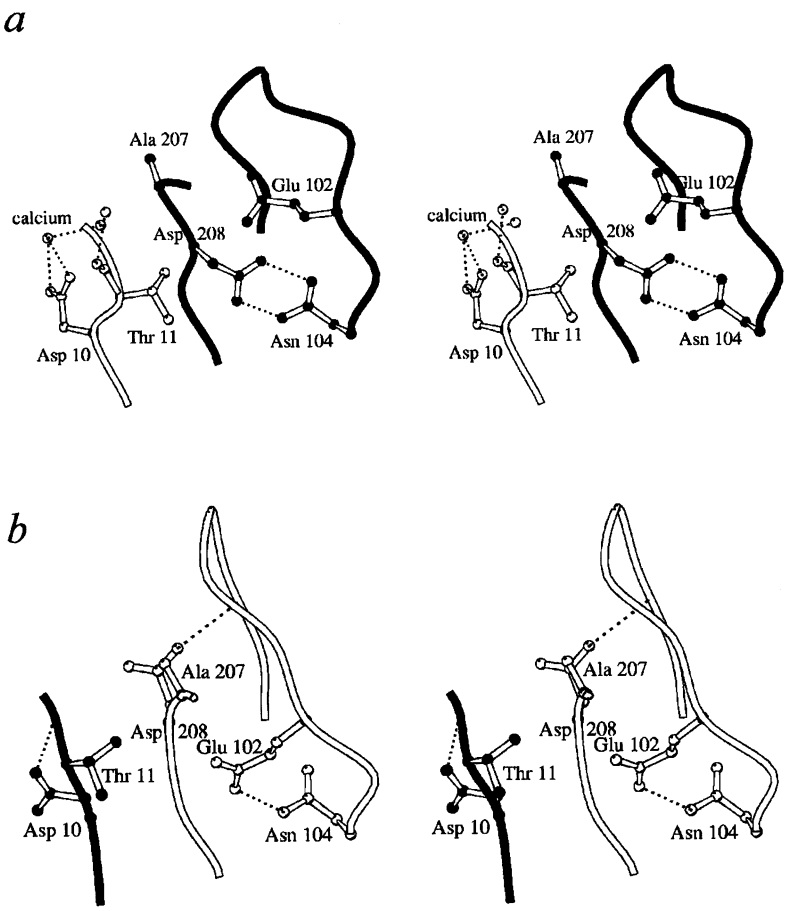


Figure 12 *Mosaic models combining the conformational features of the locked and the unlocked state of concanavanin A. The backbone is coloured black for the unlocked state and white for the locked state. (a) With structured metal binding sites as in metal-bound concanavanin A and a trans Ala207–Asp208 peptide bond, a clash is generated between the side chain of Thr11 and the backbone of the Ala207–Asp208 peptide. (b) The combination of the metal binding sites of unlocked concanavanin A with a cis Ala207–Asp208 peptide lacks the interactions that induce and maintain the cis-peptide bond*
(Reproduced with permission from Bouckaert *et al.*[52])

In common globular proteins, the native form is in its most stable state. In contrast, each native form exists in a metastable state in inhibitory serpins (serine protease inhibitors) and some viral membrane fusion proteins (see also ref. 156). Metastability in these proteins is critical to their biological functions. Mutational analyses and structural examination have previously revealed unusual interactions, such as side chain overpacking, buried polar groups and cavities as the structural basis of the native metastability. Characterization of cavity-filling mutations of α_1-antitrypsin, a prototype serpin, has been reported.[55] Figure 14

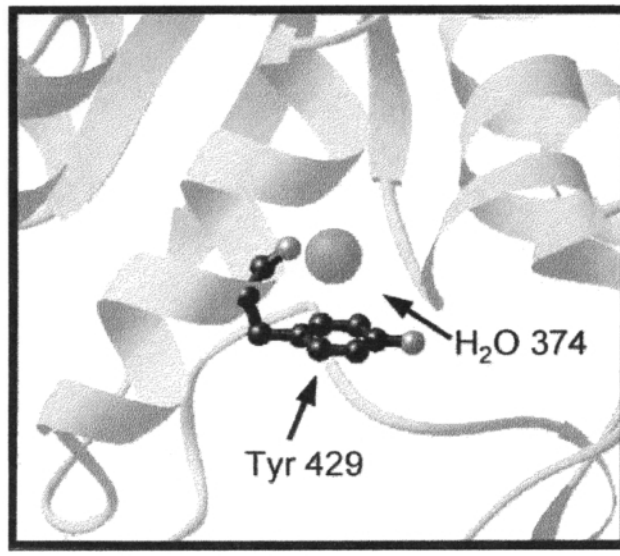


Figure 13 *Water molecule controversially assigned in the crystal structure of tryptophanase* (Reproduced with permission from De Wall *et al.*,[53] copyright 2000, National Academy of Sciences, USA)

shows α_1-antitrypsin, focusing on the interactions that occur at Gly117 with nearby amino acid side chains (Figure 56 and later figures are relevant to the present discussion). As a result of this study of a series of α_1-antitrypsin mutants, the conformational stability of the molecule is shown to increase linearly with the van der Waals volume of the side chains.

The structure of a serpin–protease complex has been determined to show that the 'inhibition by deformation' theory is justified. This remains a controversial view currently; it maintains that the mechanism of protease inhibition by serpins and by other inhibitors depends on a profound change in conformation inflicted on the enzyme. (Figures 15–17).[56]

6.3 Prion Proteins (see also Section 10.1). – The NMR structures of the recombinant 217-residue polypeptide chain of the mature bovine prion protein [bPrP(23–230)] and a C-terminal fragment, bPrP(121-230), include a globular domain extending from residue 125 to residue 227, a short flexible chain end of residues 228–230, and an N-terminal flexibly disordered 'tail' comprising 108 residues for the intact protein and four residues for bPrP(121–230), respectively (Figures 18, 19). The globular domain contains three α-helices comprising the residues 144–154, 173–194 and 200–226, and a short antiparallel β-sheet comprising the residues 128–131 and 161–164. The best-defined parts of the globular domain are the central portions of the helices 2 and 3, which are linked by the only disulfide bond in bPrP.[57]

There are differences between bovine and human prion proteins in the surface

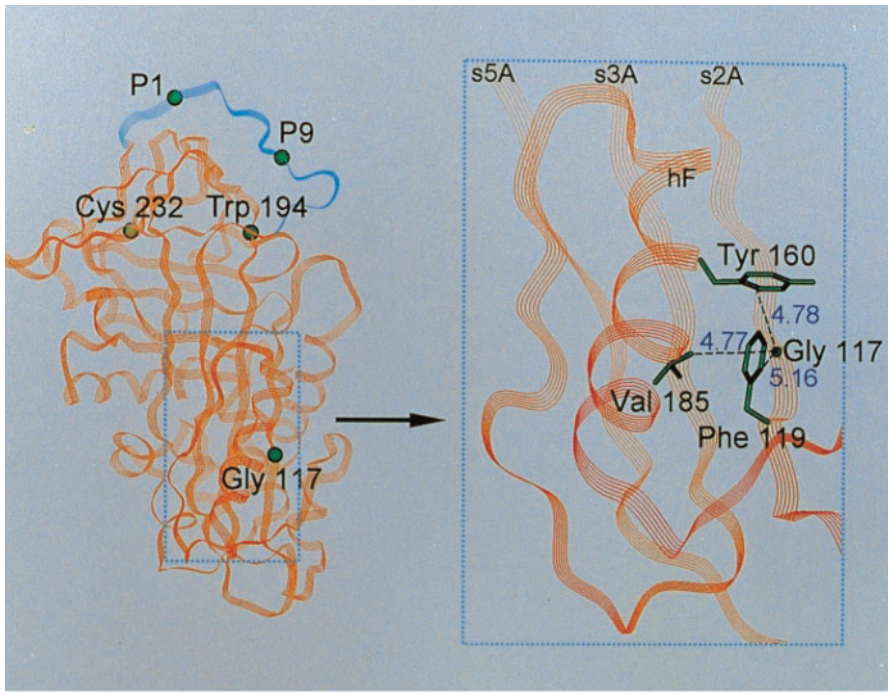


Figure 14 *(Left) Schematic drawing of the structure of α_1-AT and the cavity interactions near Gly117. The reactive centre loop, which is inserted into β-sheet A as the fourth strand upon cleavage by target protease, is shown in cyan colour. P1 and P9 are the first and ninth residues amino-terminal of the scissile bond. (Right) Cα of Gly117 and the side chains of neighbouring residues are shown in a close-up view. Interatomic distances are designated in Å and straight dotted lines*
(Reproduced with permission from Lee *et al.*[55])

distribution of electrostatic charges. This then appears to be the principal structural feature of the 'healthy' PrP form that might affect the stringency of the species barrier for transmission of prion diseases between humans and cattle.

6.4 Surface Proteins. – The particular interest in a surface protein is linked to the role that it exerts on behalf of the cell or organism. This is well established in terms of the immune response that is a crucial feature of interactions between different organisms and numerous papers have appeared in this context. However, novel contexts are being found for studies focusing on surface proteins, and new researches are finding that bacterial surface proteins are a particularly productive topic. For discussion of recent work for pili proteins, see ref. 37.

Certain pathogens possess the means by which to induce assembly of the phagocytic machinery necessary to bring about their own uptake by another organism. Among these pathogens is the facultative intracellular bacterium *Listeria monocytogenes*, a cause of meningitis and abortion in humans; this bacterium induces its own phagocytosis in a large number of nonphagocytic cell types *in vitro* through the actions of the bacterial surface proteins InlA and InlB

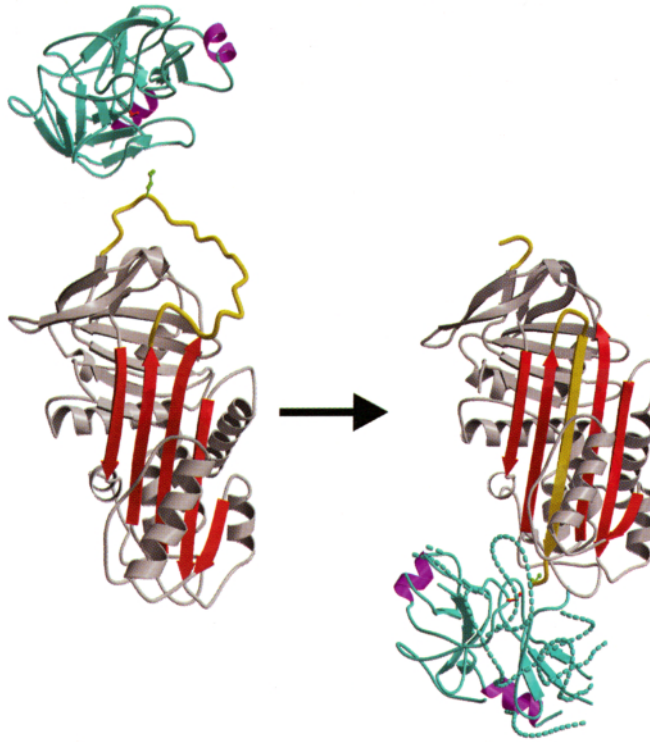


Figure 15 Native α_1-*antitrypsin with trypsin aligned above it in the docking orientation (left) and of the complex showing the 71A shift of the P1 methionine of* α_1-*antitrypsin with full insertion of the cleaved reactive-centre loop into the A-sheet (right). Red,* α_1-*antitrypsin* β-*sheet; yellow, reactive-centre loop; green ball-and-stick, P1 methionine; cyan, trypsin (with helices in magenta for the purposes of orientation); red ball-and-stick, active serine 195*
(Reproduced with permission from Huntington *et al.*[56] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

(internalin and internalin B, respectively).

The internalins are the largest class of bacterial proteins containing leucine-rich repeats (LRR), a motif associated with protein–protein interactions. Understanding the significance of the leucine-rich repeats of the *Listeria* internalins has required a long and detailed study, recent progress being represented by interpretation of the newly-determined X-ray crystal structure of the InlB LRR domain.

To exert its role, InlB causes phosphorylation of tyrosine residues of host cell adaptor proteins, activation of phosphoinositide 3-kinase and rearrangements of the actin cytoskeleton. These events lead to phagocytic uptake of the bacterium by the host cell. InlA is another surface protein of the *Listeria* internalins involved in host cell invasion, and similar interpretations have been provided for the mode of action of this protein (Figure 20).

The LRR motif is found in a functionally diverse array of proteins, including

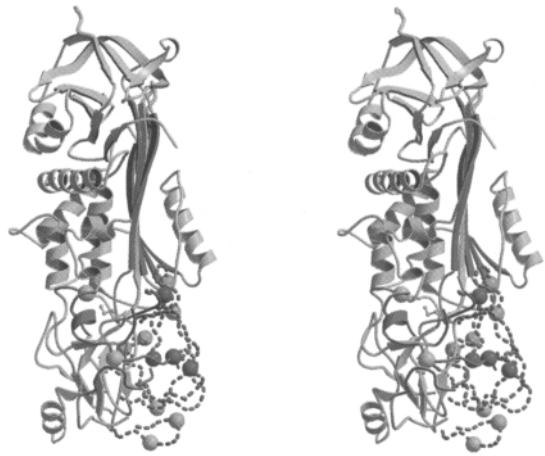


Figure 16 *Stereo view from the side of the trypsin–α_1-antitrypsin complex. The nine sites of proteolytic cleavage are shown as balls and all occur in regions of crystallographic disorder or high mobility. Cleavage sites: green, of trypsin by trypsin; yellow, of chymotrypsin by chymotrypsin; magenta, of chymotrypsin by neutrophil elastase*
(Reproduced with permission from Huntington *et al.*[56] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

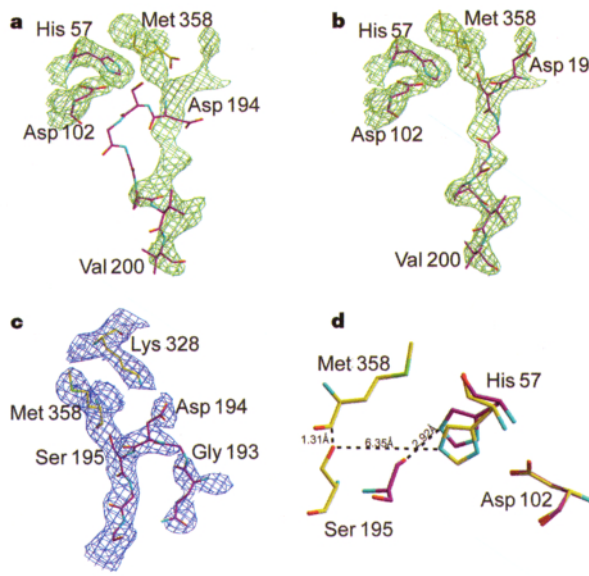


Figure 17 *Distortion of the active site of trypsin (a) initial (b) final models of trypsin and the Met358 replacement analogue. The molecular replacement solution of α_1-antitrypsin is shown in green. (c) Refined electron density (blue) shows stretching of the active site loop resulting in the loss of the oxyanion hole and the replacement of the stabilizing 'activation' salt-bridge between the N-terminal amino group and Asp194 of trypsin with Lys328 of α_1-antitrypsin. (d) The catalytic triad of native trypsin (magenta) is grossly distorted in the complex (yellow) with a shift of Ser195 away from His57 to well beyond hydrogen-bonding distance*
(Reproduced with permission from Huntington *et al.*[56] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

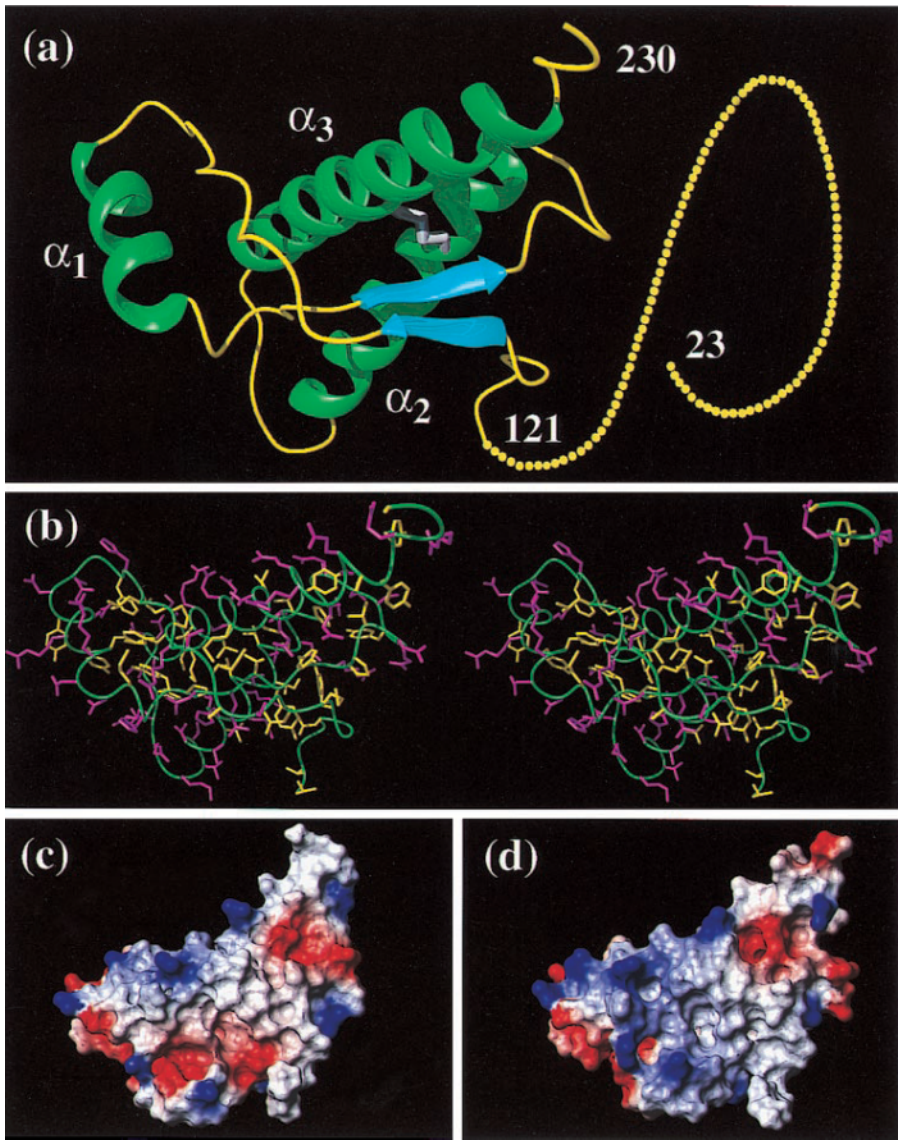


Figure 18 *(a) Intact bovine prion protein bPrP(23–230). Helices are green, β-strands are cyan, segments with non-regular secondary structure within the C-terminal domain are yellow, and the flexibly disordered 'tail' of residues 23–121 is represented by 108 yellow dots, each of which represents a residue of the tail. (b) Stereoview of an all-heavy atom presentation of the globular domain in bPrP(23–230), with residues 121–230, in the same orientation as in (a). The backbone is shown as a green spline function through the Cα positions, hydrophobic side-chains are yellow, and polar and charged side chains are violet. (c) and (d) Surface views of the globular domains of bPrP and hPrP, respectively. The orientation of the molecule is slightly changed relative to (a), so that residue 186 is approximately in the centre. The electrostratic surface potential is indicated in red (negative charge), white (neutral), and blue (positive charge)*
(Reproduced with permission from Garcia *et al.*[57] copyright 2000, National Academy of Sciences, USA)

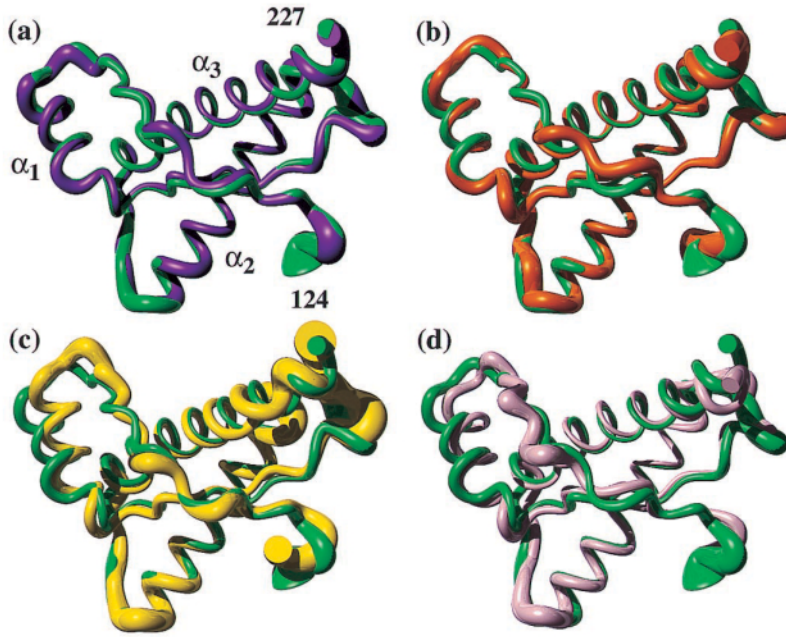


Figure 19 (*a*) *Superposition of the mean NMR structures of the sequence* 124–227 *in bPrP*(23–230)(*violet*) *and bPrP*(121–230)(*green*). (*b*)–(*d*) *Superposition of the segment* 125–227 *in bPrP*(121–230)(*green*) *with the corresponding residues in hPrP*(121–230) (*b*; *orange*), *mPrP*(121–231) (*c*, *yellow*), *and shPrP*(90–231) (*d*; *pink*), *respectively*
(Reproduced with permission from Garcia *et al.*[57] copyright 2000, National Academy of Sciences, USA)

those involved in the plant immune system and in the mammalian innate immune response.[58]

6.5 Rare Folding Motifs within Proteins. – The handful of well-known regular structures adopted by the polypeptide backbone within proteins[59] is occasionally augmented by a small number of much less common variants, some of which have featured in the current literature.

6.5.1 π-Helix. Only ten confirmed structures of the rare π-helix are listed in the Protein Data Bank, to which another example has now been added. This feature is structured with hydrogen bonding between *n*th and (n + 5)th residues within a polypeptide chain, rather than between *n*th and (n + 4)th residues as in the α-helix. It has also been labelled the 4.4_{16}-helix, following nomenclature used for the more familiar 3_{10}-helix (β-turn).[60]

6.5.2 β-Roll. A β-roll has been identified in 'RTX-toxins' ['Repeats in ToXins'; toxic proteins with repeated short glycine-rich peptide sequences such as -Gly-Gly-'X'-Gly-'X'-Asp-'X'-Leu-(or -Ile- or -Phe-)'X'-]. An alkaline protease from *Pseudomonas aeruginosa* shows this motif, which is stabilized by calcium ions bound into turns connecting the β-strands (Figure 21).[61]

Figure 20 *Structure of the InlB LRR region (residues 77–242). The right-handed coil of the LRR alternates between β-strands and 3_{10}-helices. The β-strands form the concave face of the molecule and have a superhelical twist not observed in other LRR proteins*
(Reproduced with permission from Marino *et al.*,[58] copyright 2000, National Academy of Sciences, USA)

6.5.3 The β-Helix and Stacked Parallel β-Sheets as Constituents of Antifreeze Proteins. There are now two solved structures for insect antifreeze proteins, showing unusual folding that accounts for water binding so as to form the ice structure and thereby explain the antifreeze property. The small (8.4 kDa) antifreeze protein from the beetle *Tenebrio molitor* has a tandem 12-residue repeat structure -(Thr-Cys-Thr-'X'-Ser-'X'-'X'-Cys-'X'-'X'-Ala-'X'-)$_n$ that generates a regular β-helix conformation (Figures. 22, 23).[62]

The antifreeze protein of the spruce budworm *Choristoneura fumiferana* consists of stacked parallel β-sheets.[63]

6.5.4 Knots. Knots within proteins are a source of fascination, especially from the aspect of biogenesis – how is the knot introduced in the course of protein biosynthesis? Application of a novel simulation method for pursuit of the polypeptide backbone in a protein, through assessment of known protein structures, has been described. A plant acetohydroxy acid isomeroreductase (Tyvel) has been shown on this basis to contain an unprecedented figure-of-eight knot.[64] A cystine knot in a glycoprotein α-subunit is depicted in Figure 55.

6.5.5 Other Unusual Folds in Proteins. Frataxin is a mitochondrial protein that is encoded by defects in the Friedreich ataxia gene. Frataxin causes Friedreich

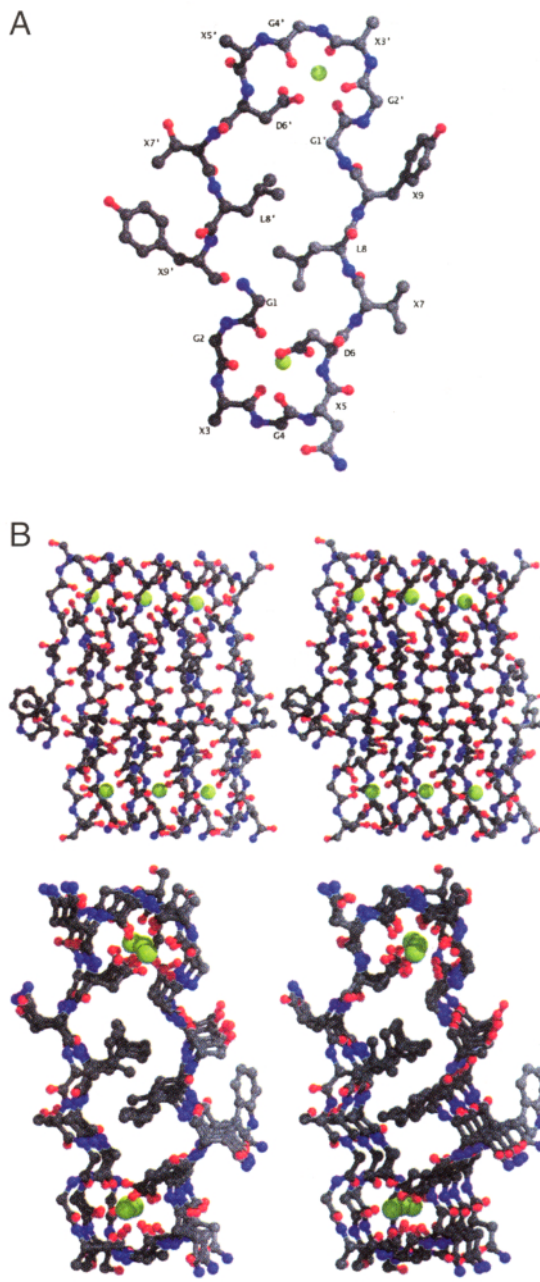


Figure 21 *The parallel β-roll. (A) One turn of the β-roll. Residues of the first nine-residue motif are labelled G1 G2* etc., *while the residues of the next motif have a prime G1′, G2′* etc. *If G1 is at position* n *in the sequence and G2 is in position (*n + *1), then G1′ is at position (*n + *9) and G2′ at position (*n + *10) and so on. (B) Two orthogonal views of the* β-roll found in the protease from P. aeruginosa. *Calcium ions are shown as balls sandwiched between the chains*
(Reproduced with permission from Lilie *et al.*,[61] *FEBS Lett.*, Blackwell Science).

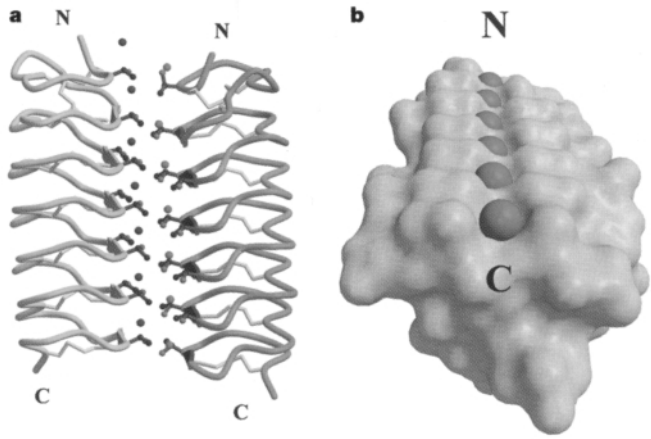


Figure 22 *Dimer of* Tenebrio molitor *antifreeze protein; (a) stabilization by hydrogen bonding to two ranks of ordered water molecules (b) surface of* Tenebrio molitor *antifreeze protein showing regular ordering of bound water molecules and showing the flat nature of the resulting surface when one rank of the water molecules is in place*
(Reproduced with permission from Liou *et al.*[62] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

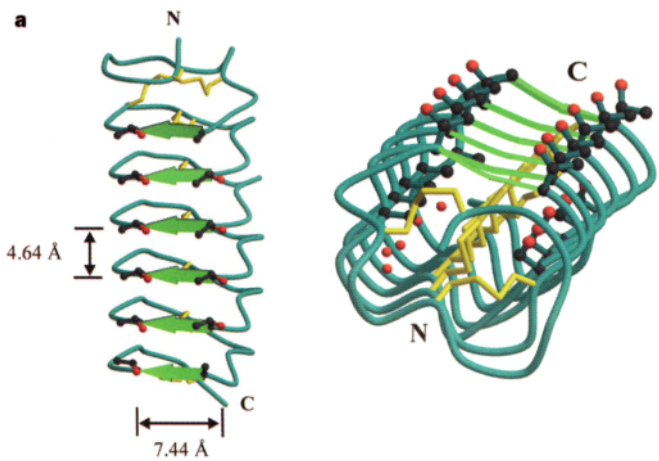


Figure 23 Tenebrio molitor *antifreeze protein. (a) Side view of β-helix and (b) end-on view. Threonine side chains present outwards from the helix and cystine disulfide bonds can be seen within the backbone, near the N-terminus The flatness of the β-sheet is assisted by the opposite side being pulled inwards, and repulsions between the threonine side chains*
(Reproduced with permission from Liou *et al.*[62] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

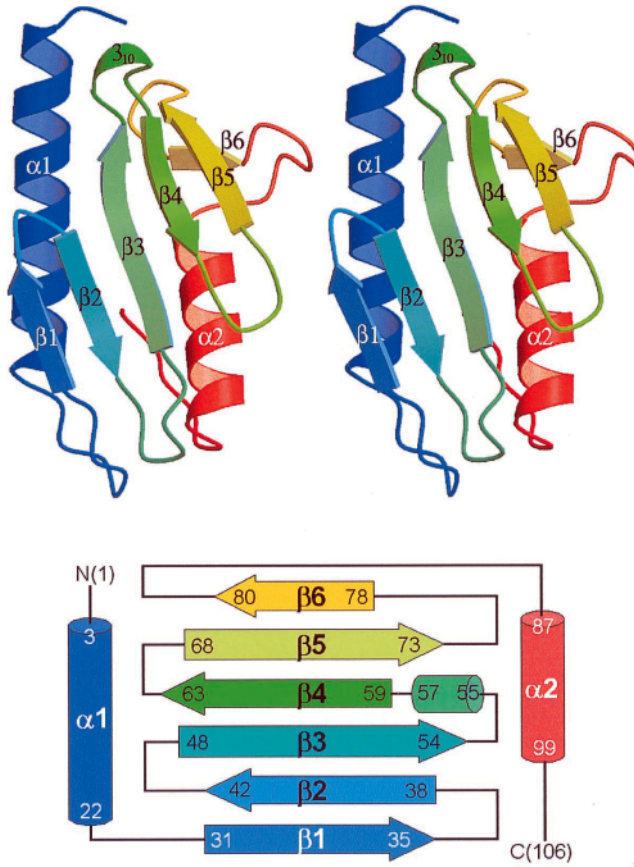


Figure 24 *Overall fold of* E. coli *CyaY. (Upper) Stereo ribbon diagram showing the secondary structure elements. Six β-strands (arrows), two α-helices (ribbons) and a 3_{10}-helix are labelled. (Lower) Topology diagram*
(Reproduced with permission from Cho *et al.*,[65] copyright 2000, National Academy of Sciences, USA)

ataxia, an autosomal recessive neurodegenerative disease and frataxin homologues have been identified in mammals, worms, yeast and bacteria.

The CyaY proteins of γ-purple bacteria are believed to be closely related to the ancestor of frataxin, and this context encouraged the effort to be made to determine the crystal structure of the CyaY protein from *Escherichia coli* at 1.4 Å resolution. This reveals a previously unidentified fold consisting of a six-stranded antiparallel β-sheet flanked on one side by two α-helices, a fold that is likely to be shared by all members of the conserved frataxin family. This structure (Figure 24) provides a starting point for the interpretation of disease-associated mutations in frataxin and for understanding the possible functions of this protein family.[65]

7 Adhesion and Binding Studies

7.1 Textbooks and Monographs. – Textbooks cover protein–protein interactions,[66] protein–protein recognition[67] and RNA-binding proteins.[68]

7.2 New Results from Binding Studies. – These studies involve either (a) protein–protein interactions or (b) protein complex formation with non-protein species. The first of these categories is covered here but there are several relevant papers located in other sections of this chapter.

New knowledge on protein–protein interactions for individual organisms, obtained through interpretation of complete genome sequences, is starting to appear in the literature. A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*, whose genome data were reported in April 1996, has been published.[69]

7.2.1 Reversible Dimerization. In the course of protein engineering studies on human FKBP, a single point mutation in the ligand-binding site (Phe36 to Met36) was discovered to convert the normally monomeric protein into a ligand-reversible dimer. The mutant (FM) forms discrete homodimers with micromolar affinity that can be completely dissociated within minutes by the addition of monomeric synthetic ligands. These unexpected properties form the basis for a 'reverse dimerization' regulatory system involving FM fusion proteins, in which association in the ground state and addition of ligand abolishes interactions. This offers the basis for constructing a 'reverse dimerization' system that should be broadly applicable as a disaggregation switch for intracellular processes.[70]

7.3 Protein–Protein Interactions Involving Chaperones. – The term 'chaperone' is often used to describe proteins that protect other proteins from stress-induced aggregation. They are therefore at the forefront of research into diseases caused by protein denaturation. Studies with α-crystallin (see also ref. 51) illustrate the numerous stages that are involved in this important type of protein–protein interaction. The chaperone binds and acts cooperatively with other heat-shock proteins to renature the stabilized and partly-denatured protein in an ATP-dependent mode. Where the denatured protein is an enzyme, some far-reaching consequences can be expected. Thus, denatured α-crystallin (see also ref. 51) is incapable of catalytic activity alone, but with the assistance of other chaperones there is recovery of function.[71] α-Crystallin does not recognize stable molten globule states of cytosolic proteins and becomes involved only with proteins that are otherwise on an irreversible path to aggregation.[72]

Complexes formed by α-crystallin have been described, a recent example involving ε-crystallin (*alias* lactate dehydrogenase B_4) isolated from duck eye lens.[73]

7.4 Metallochaperones. – Another subdivision of the chaperone family covers metallochaperones, proteins that ensure the safe delivery of metal ions that could

otherwise enter into adventitious reactions or other mischief at various unintended binding sites.[74]

7.4.1 Case Studies in Chaperones. The effects of molecular chaperones and folding catalysts on the folding and subunit assembly of heterodimeric luciferase after *de novo* synthesis of subunits in rabbit reticulocyte lysate have been determined.[75]

ClpA, a bacterial member of the Clp/Hsp100 chaperone family, is an ATP-dependent molecular chaperone and is the regulatory component of the ATP-dependent ClpAP protease. Its behaviour has been studied in some detail in a recent study of the mechanism of binding and unfolding of proteins by ClpA and translocation to ClpP, a fusion protein that joins the ClpA recognition signal from RepA to green fluorescent protein (GFP).

This was studied as a model substrate and revealed a clear sequence of events. ClpAP degrades the fusion protein *in vivo* and *in vitro*, the substrate binding specifically to ClpA in a reaction that requires ATP binding but not ATP hydrolysis. Binding alone is not sufficient to destabilize the native structure of the GFP portion of the fusion protein. Upon ATP hydrolysis the GFP fusion protein is unfolded, and the unfolded intermediate can be sequestered by ClpA if a non-hydrolysable analogue is added to displace ATP. ATP is required for release.

Although ClpA is unable to recognize native proteins lacking recognition signals, including GFP and rhodanese, it interacts with those same proteins when they are unfolded. Unfolded GFP is held in a non-native conformation while it is associated with ClpA, and its release requires ATP hydrolysis. Degradation of unfolded untagged proteins by ClpAP requires ATP even though the initial ATP-dependent unfolding reaction is bypassed. These results suggest that there are two ATP-requiring steps: an initial protein unfolding step followed by translocation of the unfolded protein to ClpP, or in some cases, release from the complex.[76]

7.5 Proteins Complexed with Non-protein Species. – Saxiphylin, from the plasma of the North American bullfrog *Rana catesbiana*, is a saxotonin-binding protein with two thyroglobulin Type I domains that has broader activity as an inhibitor of papain-like cysteine proteases.[77] Inhibition by proteins requires a preliminary binding step or steps involving the protein inhibitor and an active site domain of the enzyme. A newly discovered insect immunoglobulin binds insulin and related peptides and inhibits their activities.[78]

The details of the binding geometries shown in several recent examples are usually deduced from crystallographic analysis or NMR spectra. Three C-terminal residues of intestinal fatty acid binding protein participate in four main-chain hydrogen bonds and two electrostatic interactions to sequentially distant backbone and side chain atoms (Figure 25).[79] These three residues are key elements controlling late folding events in the development of the native conformation of this protein.

Troponin-C and troponin-I of skeletal muscle participate in a critical molecu-

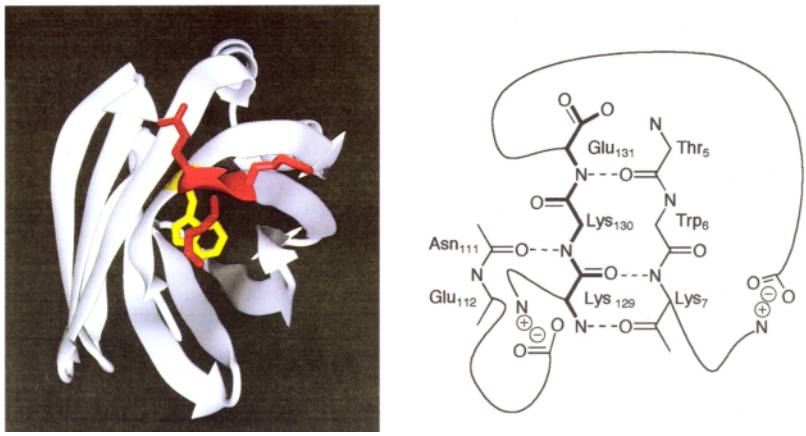


Figure 25 *(Left) IFABP showing the orientation of side chains 129-131 and their contact with sequentially distant β-strands. (Right) Hydrogen bonds and salt-bridges that are disrupted by truncation in W6F IFABP*$_{1–128}$
(Reproduced with permission from Clerico et al.[79] and Elsevier Science)

lar switch for calcium-dependent regulation of the contractile mechanism, which involves a β-hairpin of troponin-I as the inhibitory region.[80] Secretagogin is a novel Ca^{2+}-binding protein[81] (calcium-binding proteins are also referred to in refs. 61, 182).

Endothelial differentiatioactor I binds calmodulin, and therefore this protein may have relevance to angiogenesis.[82] A new calmodulin-type protein (given the name calmodulin-like skin protein) from human epidermis has a possible role in the regulation of events in late keratinocyte differentiation. It is capable of binding with calcium and calmodulin to expose hydrophobic parts that probably interact with target proteins.[83]

Interactions between cytochrome f and plastocyanin have been determined (Figure 26).[84]

Immune response involves an initial binding of antigen to cellular Fc receptors, followed by various cellular effector functions of the immune system. Structures of complexes of human IgG1 Fc fragment with FcγR111 have been determined (Figures 27–29)[85] and also the corresponding complex of FcεR1α with the Fc fragment of human IgE,[86] have been determined. These provide a model for the immune complex recognition process.

Crystal structure determination of fibroblast growth factor receptor ectodomain bound simultaneously to a ligand and to a heparin disaccharide has been reported. It shows the molecular architecture of the complex, centred around the heparin molecule. A structural basis for the essential role of heparin sulfate in FGF signalling is provided through this study.[87]

Species that have become known as chemokines (*chemo*attractant cyto*kines*), are generally small (5–20 kDa) basic proteins that are involved in heparin binding and leucocyte-directing activities. New examples include fractalkine,

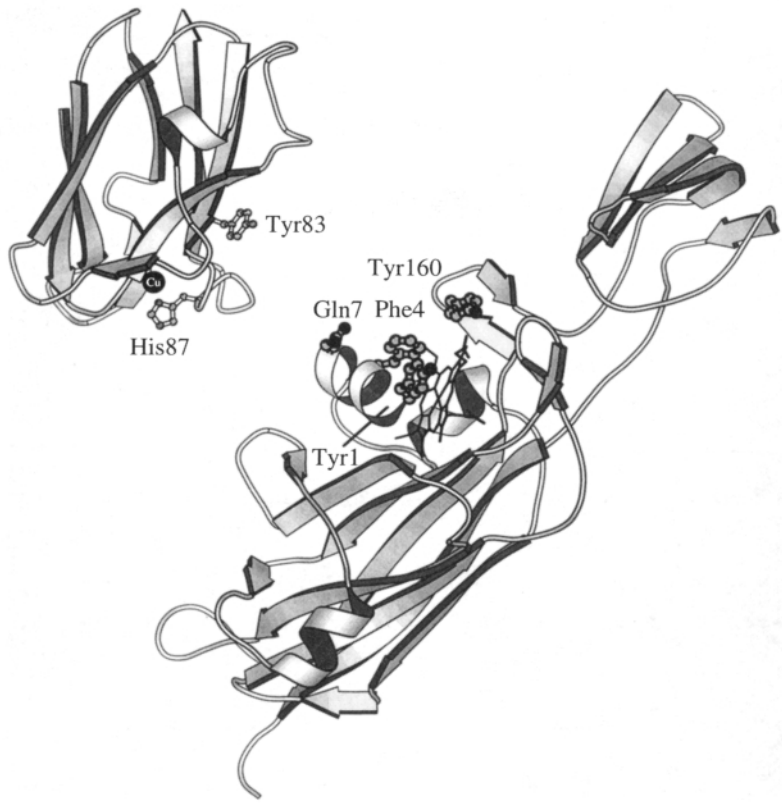


Figure 26 *Turnip cytochrome f and pea plastocyanin. The side chains of Tyr83 and His87 of plastocyanin and the side chains of the aromatic shield residues Tyr1, Phe4 and Tyr160 and the invariant Gln7, of cytochrome f, are shown*
(Reproduced with permission from Gong *et al.*,[84] *Eur. J. Biochem.*, Blackwell Science)

notable for its novel quaternary arrangement (Figures 30, 31).[88]

7.6 Lectins. – Lectins are continuing to grow in stature, because of their roles in several important contexts, exerting effects through binding, *e.g. Diocleinae Grandiflora* lectins bind trimannosides concanavalin A and 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannose (this is found in the core region of all asparagine-linked carbohydrates).[89] X-Ray crystallographic study shows that an unusual carbohydrate binding site (Figure 32) is a feature of the complex of leukoagglutinin (one of the lectins of the legume tree *Maackia amurensis*) with sialyllactose[90] (see also ref. 52).

A mammalian peptidoglycan and its designated recognition protein show high affinity, and the resulting complex has an antibacterial function in neutrophils.[91]

7.7 Dissociation (see also Section 5.3 and ref. 108). – The opposite of folding

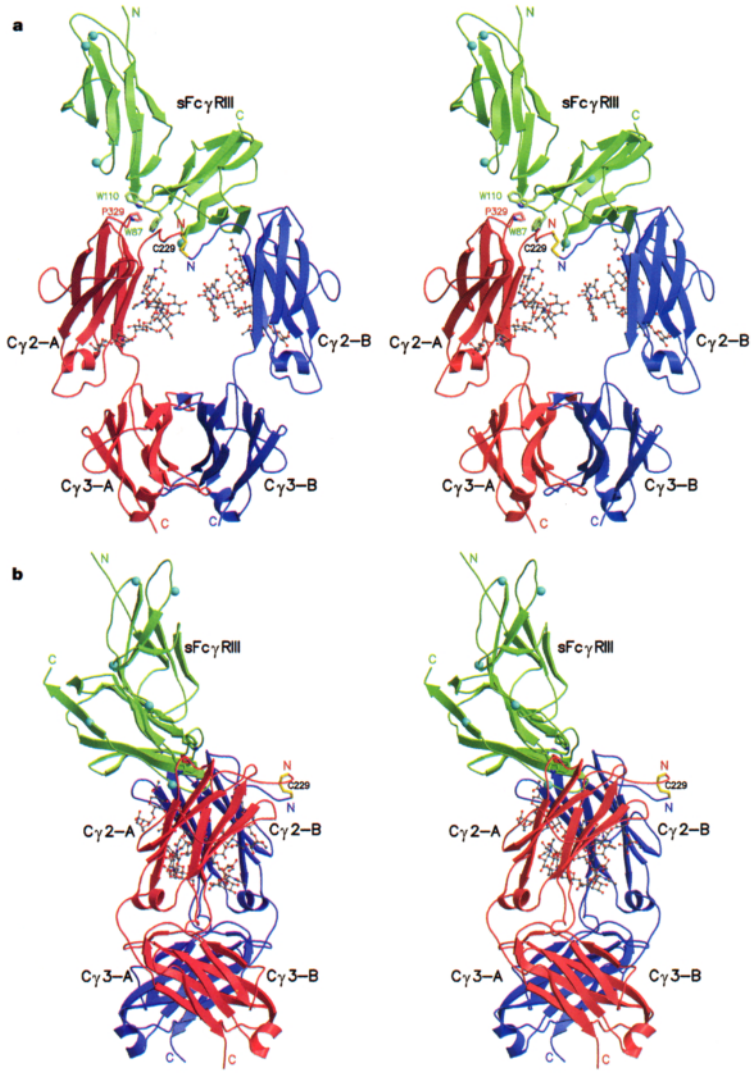


Figure 27 *Overall structure of the sFcγRIII-hFc1 complex. (a) Dimer axis of hFc1 (red and blue) oriented vertically . The proline sandwich (Pro329 of the Cγ2-A domain and Trp87 and Trp110 of sFcγRIII (green), the upper one-third of the structure) is shown in ball and stick form, together with the carbohydrate residues of the Fc fragment and the inter-chain disulfide bridge of the Cys229 residues. (b) View after rotation by 90° about the vertical axis*
(Reproduced by permission from Sondermann *et al.*[85] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

and aggregation, dissociation and denaturation, represent one of the long-established areas of study when it comes to protein behaviour. In a recent example, ferritin from *Listeria innocua* has been shown to dissociate below pH 2, a consequence of dismemberment of the hydrophilic and hydrophobic interac-

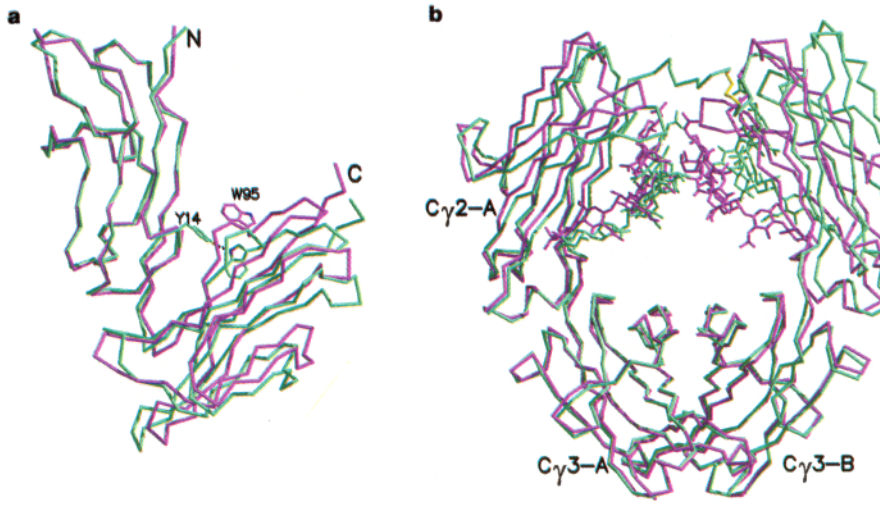


Figure 28 *Cαtrace of the complex, oriented as in Figure 27. (a) Trp95 and Tyr14 form a new hydrogen bond as a consequence of complexation and are shown in ball and stick form (the complex is cyan, and free sFcγRIII is magenta). (b) Overlay of the complex and hFc1 structures (colours as in (a))*
(Reproduced with permission from Sondermann *et al.*[85] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

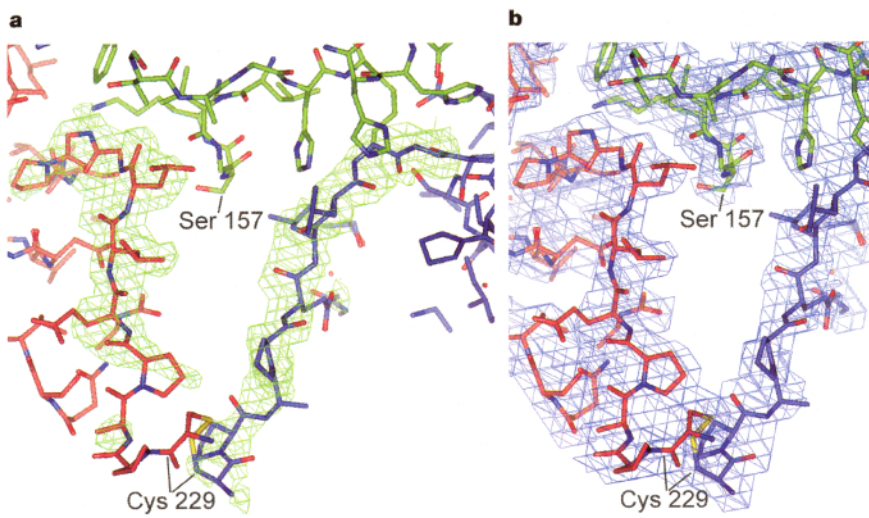


Figure 29 *Omit map of the hinge region of the complex, oriented as in Figure 27 (a) intermediate (b) final electron density map showing detail in the region of Cys229 and Ser157 (colour coding as in Figure 27)*
(Reproduced with permission from Sondermann *et al.*[85] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

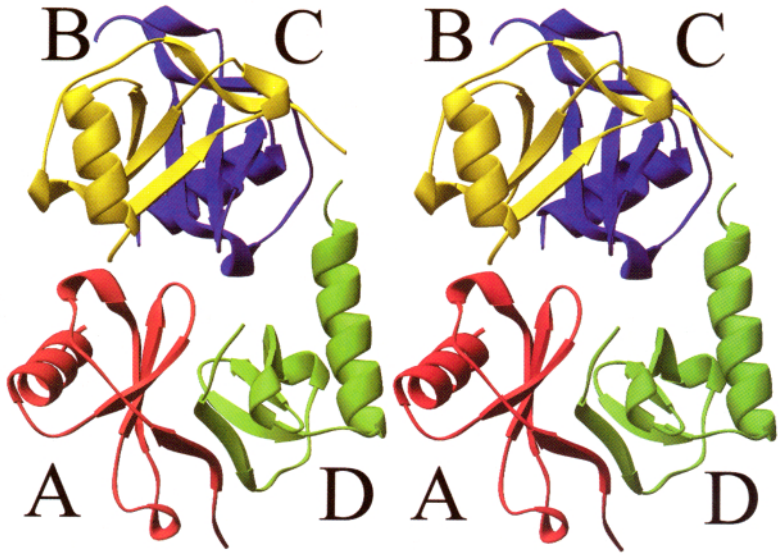


Figure 30 *Stereo ribbon drawing of CDF asymmetric unit*
(Reproduced with permission from Hoover *et al.*[88])

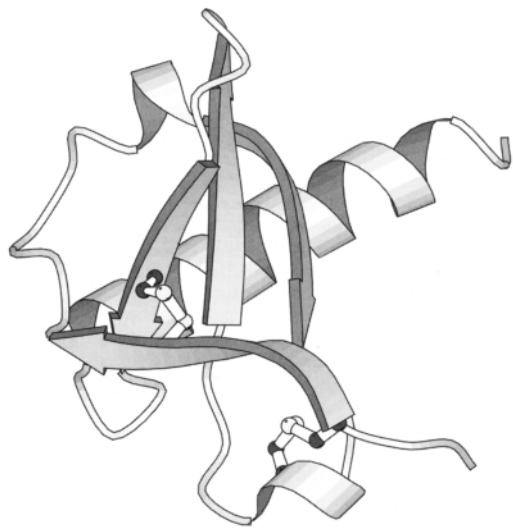


Figure 31 *Ribbon drawing of CDF monomers (monomer D). Monomer B is nearly identical to monomer D, whereas monomers A and D differ within residues 5–15. Also, residues 68–74 are ordered in monomer D producing a C-terminal extension of the α-helix by 1.5 turns*
(Reproduced with permission from Hoover *et al.*[88])

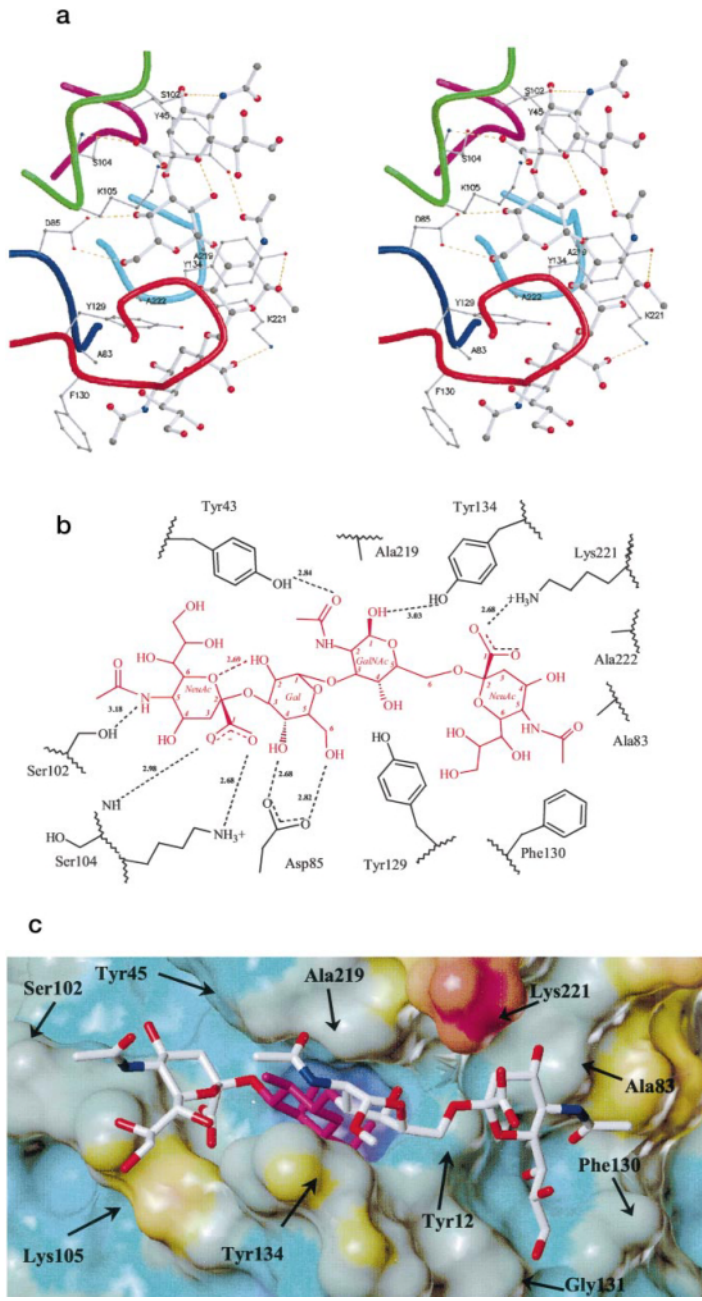


Figure 32 *Model of the interaction between MAH and the tetrasaccharide NeuAc(2-3)Gal(1-3)[NeuAc(2-6)]GalNAc. (a) Stereoview of the MAH binding. (b) Schematic representation of the modelled interaction between sialyloligosaccharide and MAH. Residues differing from the MAL binding site are boxed. (c) Connolly surface of the MAH binding site coded according to the electrostatic potential from blue (negative) to red (positive). The galactose in the monosaccharide binding site is shown in magenta*
(Reproduced with permission from Imberty *et al.*[90])

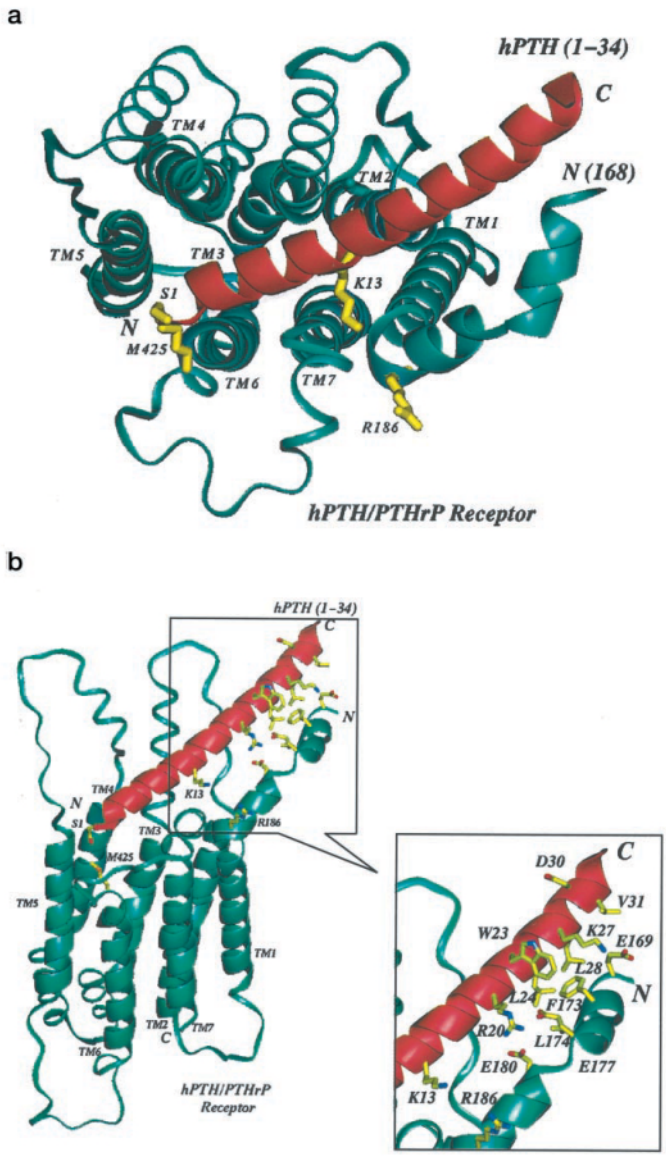


Figure 33 *Model of hPTH(1–34)(red) binding to the PTH/PTHrP receptor (blue). Residues at the ligand-receptor interface are highlighted in yellow. The sequence starting at N and ending at C is hPTH, shown sitting in the receptor crevice. (b) Side view of (a) rotated through 90° and an enlarged view of this region*
(Reproduced with permission from Jin *et al.*[93])

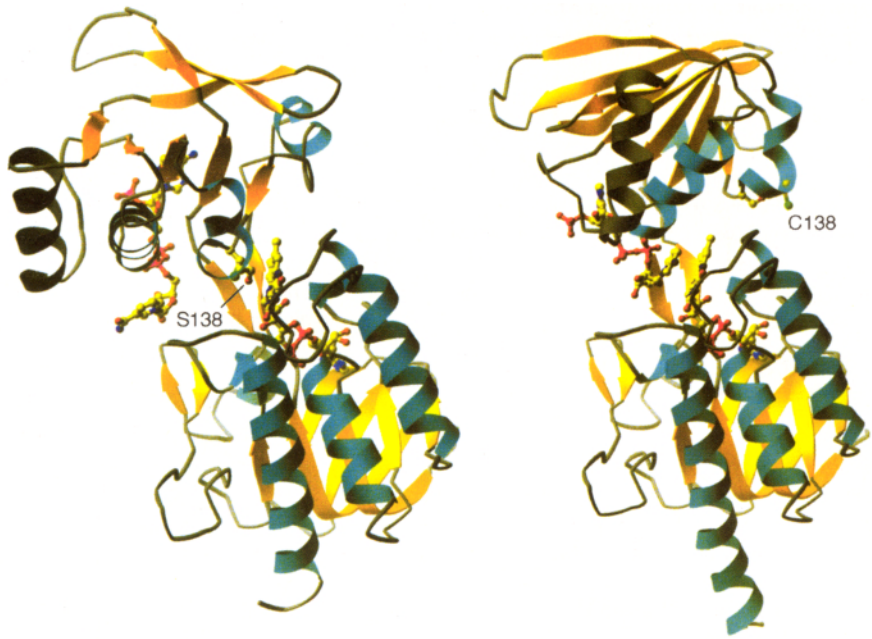


Figure 34 *Diagrams of the FO (Left) and FR (Right) conformations of thioredoxin reductase. $NADP^+$ and $AADP^+$ are bound respectively in the two structures*
(Reproduced with permission from Williams *et al.*,[96] *Eur. J. Biochem.*, Blackwell Science)

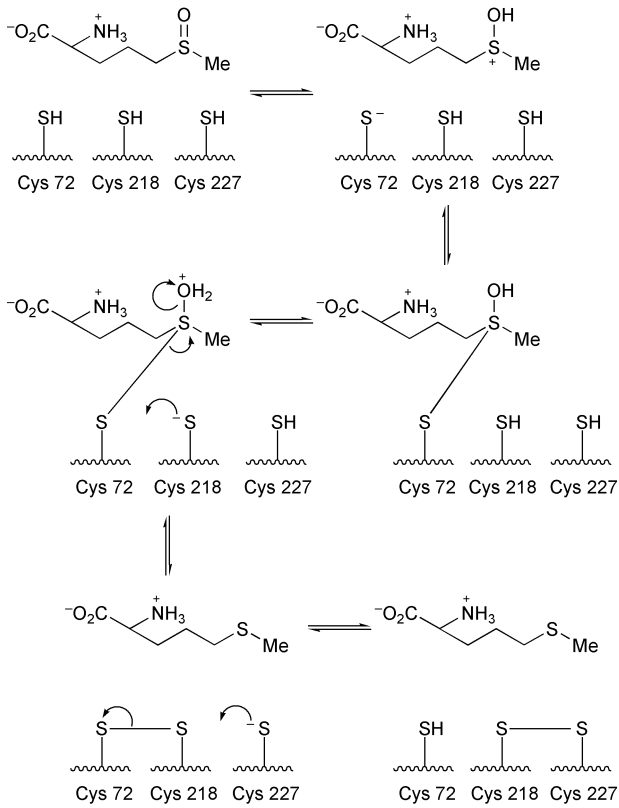
tions that sustain the dodecameric quaternary structure.[92]

7.8 Receptors. – Interaction of sequence 1–34 of human parathyroid hormone with the hormone receptor has been modelled (Figure 33), in view of the interest in this peptide that results in increased bone density formation in patients with osteoporosis.[93]

8 Enzyme Studies

8.1 Textbooks and Monographs. – Textbooks have covered proteolytic enzymes[94] and tyrosine phosphoprotein phosphatases[95] (see also refs. 1, 4, 8).

8.2 New Studies. – The two proteins comprising the thioredoxin–thioredoxin reductase system are of considerable interest as indicators of a wide variety of diseases including rheumatoid arthritis, HIV-AIDS and cancer, and their importance as prospective drug targets is therefore understandable. A series of mini-reviews has appeared covering various aspects of this enzyme system (Figure 34).[96] The first structure (NMR) of a thermostable thioredoxin supports the hypothesis for the role of long range electrostatic interactions in favouring thermostability in proteins[97] (see also ref. 129).



Scheme 2 *Involvement of cysteine residues in mode of action of peptide methionine sulfoxide reductase*
(After Lowther *et al.*[100])

Mammalian thioredoxin reductases are dimers homologous to glutathione reductase with a selenocysteine residue in a conserved C-terminal sequence -Gly-Cys-SeCys-Gly-, and selenium is required for the proper function of this enzyme, thus explaining the essential role of this trace element in cell growth.[98] A new family of omega class glutathione transferases has been found in several species, including human. The defining difference is the presence of cysteine at the active site rather than tyrosine and serine which is characteristic of all other known eukaryote glutathione transferases.[99]

Disulfide–thiol interchange is involved in catalysis by peptide methionine sulfoxide reductase, as depicted in Scheme 2.[100] Several cysteine residues are implicated and a parallel disulfide reduction operation is needed to restore the enzyme to its catalytic state at the end of each cycle.

Another enzyme, α-fetoprotein (a large serum glycoprotein used routinely to mark embryonic disorders through changes in its level in serum during pregnancy) has received an astonishing amount of attention (about 11 000 papers, roughly one published each day since 1969), but is still largely a mystery in terms of its structural features; some details of the relationship between conformational

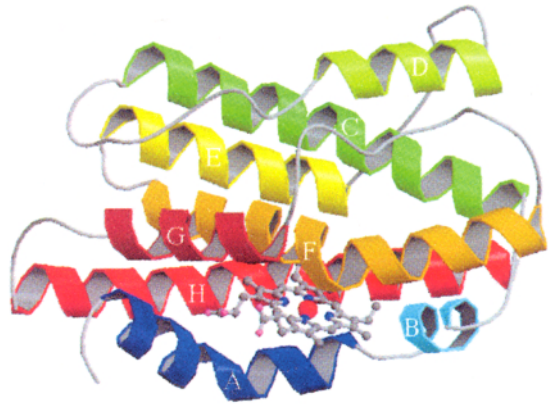


Figure 35 *Rat heme oxygenase-I heme complex showing the protein backbone and heme. Eight helices are present*
(Reproduced with permission from Sugishima *et al.*,[104] *FEBS Lett.*, Blackwell Science)

transitions in AFP and its function have emerged from a scrutiny of existing results.[101]

X-Ray studies have given structural details for the the complex formed between amylomaltose from *Thermus aquaticus* and its potent inhibitor acarbose (a maltotetraose derivative).[102]

An NMR study of the N36-boxB RNA complex has been reported. N36 is the N-terminal 36-residue peptide from the N-protein from bacteriophage λ and includes the characteristic arginine-rich motif that is involved in transcriptional antitermination of phage λ. This work demonstrates further the power of direct spectroscopic analysis in assignments of structural features to protein–RNA complexes.[103] Homonuclear and heteronuclear NMR data (2D and 3D) for three mutant peptides were interpreted to reveal the roles of particular residues in the formation of this complex. A description of rat heme oxygenase-I complexed with heme (Figures 35, 36) has appeared.[104]

X-Ray studies of the ribosome-inactivation protein, saporin SO6 from *Saponaria officinalis*, interacting with the ribosome reveal a fold in the protein that is typical of other plant toxins.[105]

Purple bacteria (*Rhodobacter sphaeroides*) engage in photosynthesis through membrane-bound reaction centres which amount to protein complexes carrying the requisite pigments and cofactors. An X-ray study of the system carrying anthraquinone in place of the essential redox component, ubiquinone-10, has been conducted so as to gain understanding of the energetics of the quinone system as a whole.[106]

Interactions have been delineated within the four subunits of polymerase ε (the first proofreading polymerase, essential for chromosomal replication, to be purified from yeast).[107] Yeast pyruvate decarboxylase complexed with its activator pyruvamide has been probed at 2.4 Å resolution by X-ray crystal analysis (Figure 37).[108]

A kinetic study that also features in this paper shows that pyruvamide triggers

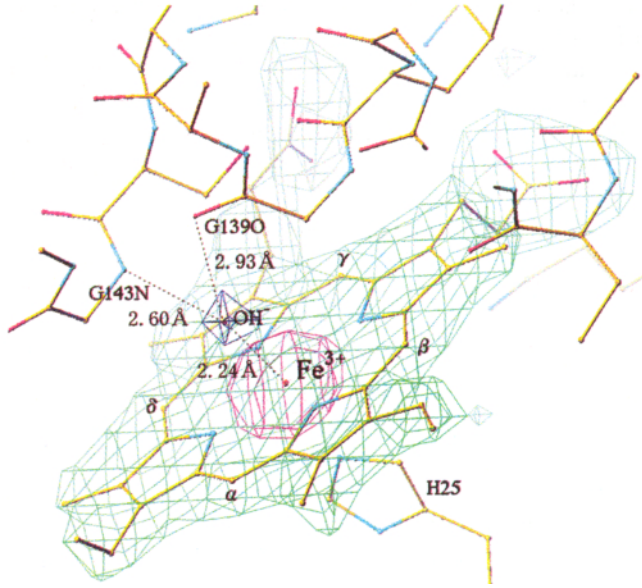


Figure 36 *Electron density near heme in rat heme oxygenase-I heme complex. View takes in the distal side of heme*
(Reproduced with permission from Sugishima *et al.*,[104] *FEBS Lett.*, Blackwell Science)

a disorder–order transition of two active-site loop regions as a key stage in the activation process.

The tertiary structure of a complex formed from caspase-9 after inhibition by Ac-Asp-Val-Ala-Asp-fluoromethyl ketone has been described (Figures 38–41).[109]

Feedback regulation of pantothenate kinase by coenzyme A has been explained on the basis of newly-clarified structural features of the system (Figure 42).[110]

Allosteric regulation of pyruvate kinase, an essential catalyst within the glycolytic pathway, has been studied through a standard site-directed mutagenesis approach (Figure 43).[111]

The membrane-associated serine protease, corin, has been established to be the long-sought pro-atrial natriuretic peptide converting enzyme (pro-ANP) responsible for releasing the biologically active cardiac hormone, ANP, within cardiac myocytes. These results follow from the recent successful cloning and use of corin, an enzyme that is highly expressed in the heart.[112] The cleavage in pro-ANP by corin is highly sequence specific. The implication is that the corin-mediated pro-ANP activation may play a role in regulating blood pressure because atrial natriuretic peptide (ANP) is essential for the regulation of blood pressure. ANP is synthesized as a precursor, pro-ANP, that is converted to biologically active ANP by an unknown membrane-associated protease.

The first three-dimensional structures of the acetylcholinesterase of fruit fly

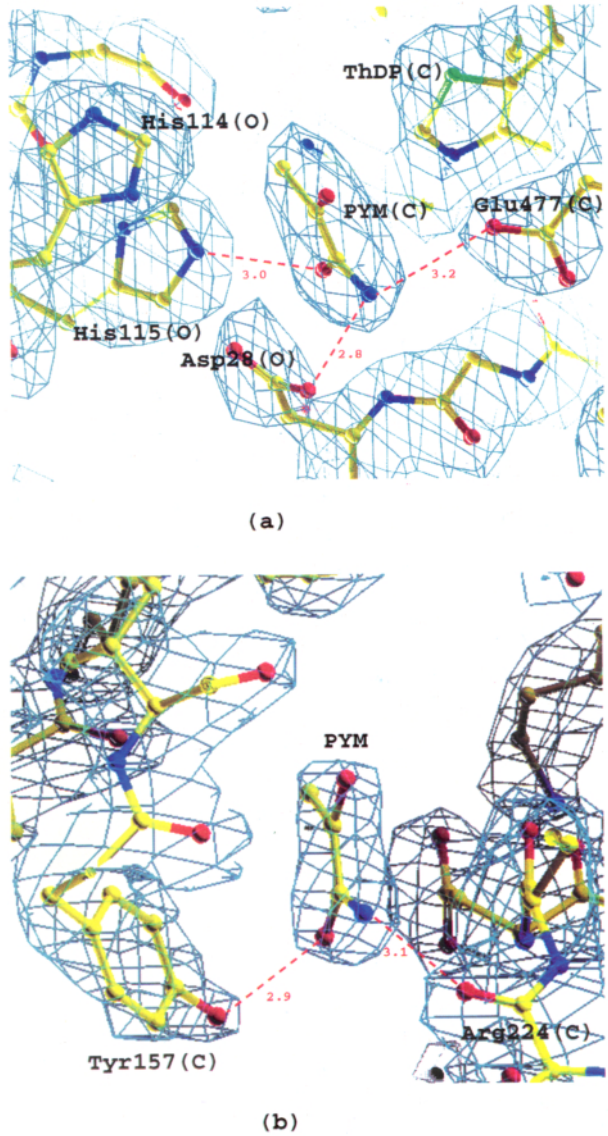


Figure 37 *Electron density at the bound pyruvamide (a) at the active site (b) at the interface between the pyruvamide domain and the R domain*
(Reproduced with permission from Lu *et al.*,[108] *Eur. J. Biochem.*, Blackwell Science)

(*Drosophila melanogaster*) and of its complexes with two potent inhibitors 1,2,3,4-tetrahydro-*N*-(phenylmethyl)-9-acridinamine and 1,2,3,4-tetrahydro-*N*-(3-iodophenylmethyl)-9-acridinamine have been reported.[113] Also the structures of alcohol dehydrogenase allozymes ADHS, ADHF, and ADHUF from *Drosophila melanogaster*.[114]

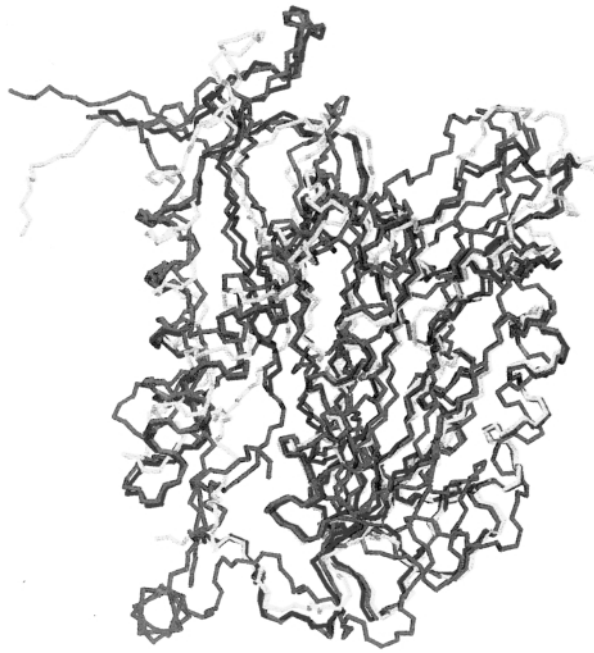


Figure 38 *Superimposed structures of caspases-1, -3, -8 and the predicted structure of caspase-9*
(Reproduced with permission from Chou *et al.*,[109] *FEBS Lett.*, Blackwell Science)

β-Galactosidase from *E. coli* has multiple metal-binding sites.[115]

Adenosine kinase from the pathogen protozoan parasite *Toxoplasma goudii* allows the organism to acquire purines from their host. This opens up targets for antiparasitic chemotherapy, since the pathogen depends on this route for life-enabling nutrients, and a structure determination of the enzyme at 1.8 Å resolution reveals structural differences from the human enzyme that were anticipated on the basis of sequence differences.[116]

The active site of formate dehydrogenase from *Candida boidinii* binds NAD^+ (Figures 44, 45).[117]

Ovotransferrin is a glycoprotein that folds into two distinct homologous lobes (N- and C-lobes). The interdomain disulfide bridge has no role in iron uptake but is important in maintaining conformation (Figure 46).[118]

Cathepsin-like cysteine proteases contain a hydrophobic stack of three tryptophan side chains, a structural feature that stabilizes prodomains.[119]

Urokinase, and plasminogen formed from it by processing, are serine proteinases whose genesis is regulated by α3β1-integrin.[120] Leucocyte β2-integrins, among the first adhesion molecules to be studied, have been reviewed.[121] Intimin and its receptor are bacterial proteins that mediate adhesion between mammalian cells and attaching and effacing pathogens, such as enteropathogenic *E. coli* which cause considerable morbidity and mortality in many parts of the world. With such an impetus, numerous studies, including X-ray structure

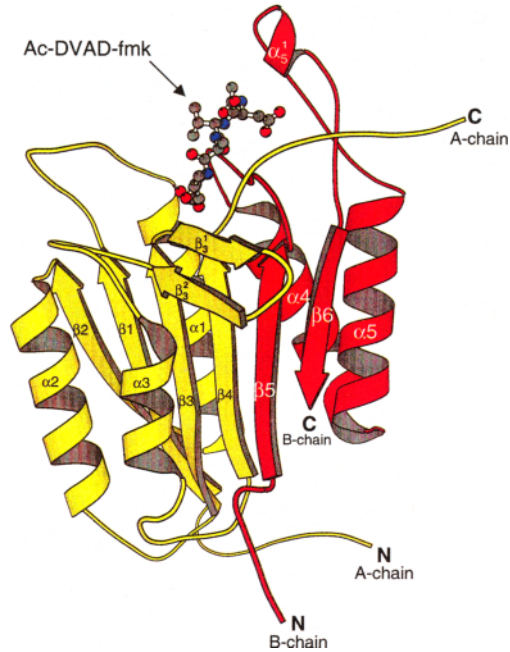


Figure 39 *Caspase-9 alkylated by Ac-Asp-Val-Ala-Asp-CH_2-F (the alkyl group is in ball and stick form at the top)*
(Reproduced with permission from Chou *et al.*,[109] *FEBS Lett.*, Blackwell Science)

determination of this complex, have been reported.[122]

The structure of a novel bacterial esterase at atomic resolution has been reported, and further details of the function of this enzyme will be needed so as to allow interpretation of its mode of action.[123]

Holliday junction resolving enzymes (metal ion-dependent endonucleases) recognize and cleave four-way DNA junctions that arise through strand exchange between homologous duplex DNA species (Figure 47). The enzyme Hjc is a member of this family that repairs branched DNA by manipulating the adjacent region into a 2-fold symmetric X-shape, as shown by a site-directed mutagenesis study, the first in this area, that assigns roles to the various catalytic residues of this enzyme.[124]

The m5C RNA and m5C DNA methyl transferases use different cysteine residues as catalysts. Notwithstanding the highly homologous sequences and similar functions, the m5C RNA methyl transferase is unusual in using a different cysteine side chain as a catalytic nucleophile than is used by the m5C DNA methyl transferase. The catalytic cysteine seems to be determined, not by the target base that is modified, but by whether the substrate is DNA or RNA. The function of the conserved ProCys sequence in the RNA m5C methyl transferases remains unknown.[125]

8.3 Newly Discovered Enzymes. – All caspases studied previously (see also

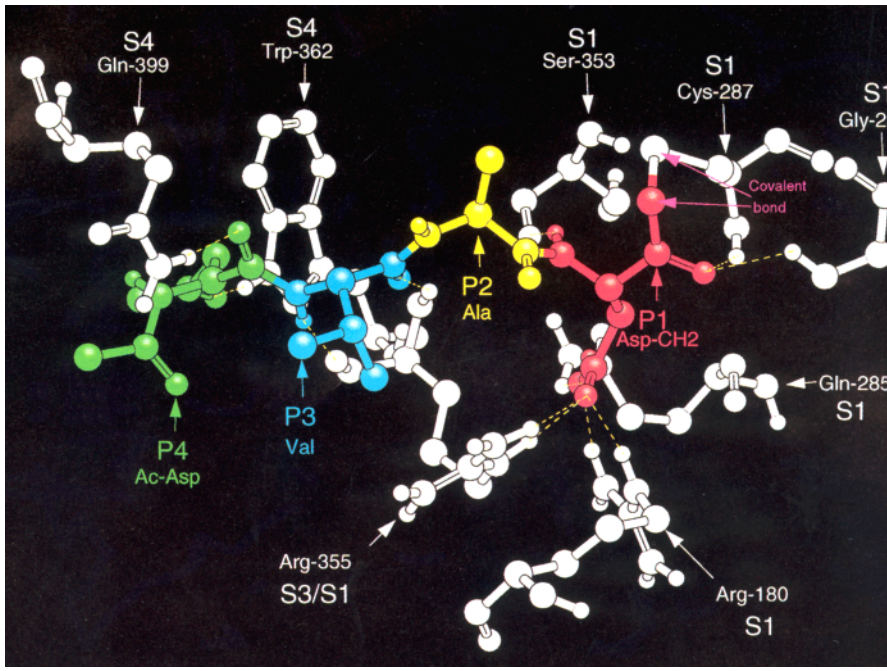


Figure 40 *Caspase-9 alkylated by Ac-Asp-Val-Ala-Asp-CH_2-F (the alkyl group is in ball and stick form at the near left) showing binding interactions with nearby groupings*
(Reproduced with permission from Chou *et al.*,[109] *FEBS Lett.*, Blackwell Science)

refs. 109, 126, 174, 176) have been shown to cleave protein substrates at the carboxy-terminal side of an aspartate residue, but a *Drosophila* caspase DRONC (one of seven caspases in this species) is unique in cleaving peptides either at a peptide bond following a glutamate or following an aspartate, depending on the nearby substrate structure.[126] A caspase eliminates a key factor that could reverse neural apoptosis by catalysing the cleavage of cAMP response element binding protein, a transcription factor that mediates the nerve growth factor survival signals.[127]

Cyclophilins from the human parasite nematode *Brugia malayi* have peptidyl-prolyl *cis–trans* isomerase activity. X-ray crystal analysis of a complex formed between one of these proteins and cyclosporin A gives insight into the reasons for the low affinity of cyclophilins for the drug, and explains the resistance to it, shown by the nematode.[128]

The first biochemical analysis of the separate stages of the condensation of indole with serine catalysed by a thermostable tryptophan synthase at high temperature has been described. The condensation involves indole 3-glycerol phosphate as a key intermediate. The study has been based on the enzyme from *Thermococcus kodakaraensis* KOD1, the first archaeal representative of this class.[129]

A serine hydrolase is a key determinant in the microbiological degradation of

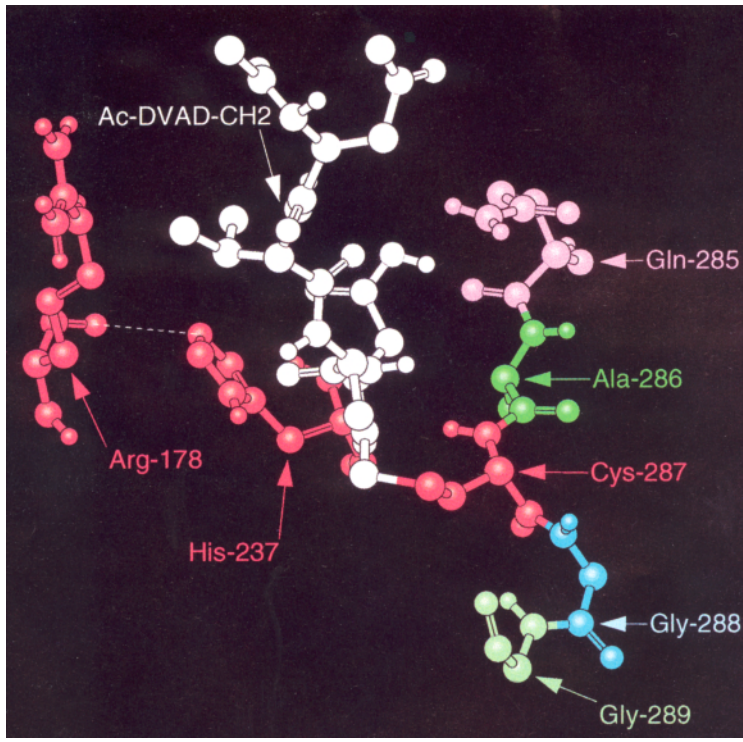


Figure 41 *Caspase-9 alkylated by Ac-Asp-Val-Ala-Asp-CH_2-F (the alkyl group is in ball and stick form at the top) showing binding interactions with nearby groupings* (Reproduced with permission from Chou *et al.*,[109] *FEBS Lett.*, Blackwell Science)

polychlorinated biphenyls.[130] These industrial chemicals persist in the environment, even though their use has been banned for more than 20 years, and it has been assumed until now that their degradation would have to depend on abiotic agencies.

β-1,3-Glucanase from ripe banana fruit closely resembles previously characterized plant β-1,3-glucanases.[131] The involvement of this enzyme (Figure 48) in the ripening and softening of the fruit was considered to be likely.

Methionine aminopeptidase structure and function have been reviewed (part of a special issue of the journal concerned with preoteolytic enzymes and their inhibitors).[132] A display of the structure of this enzyme and its interactions with a bestatin-type inhibitor is shown in Figure 49. Figure 50 summarises the structures of the five known types of this enzyme.

Cyclomaltodextrinase is a multispecific enzyme of the α-amylase family (Figure 51), that releases maltose and panose by cleavage of α-1,4-glycosidic bonds. An N-terminal extension of about 130 amino acid residues, not seen in α-amylases that are unable to deal with cyclomaltodextrin and pullulan, contributes to the active site.[133]

8.4 Mechanistic Studies. – Classical methodology continues to serve well the

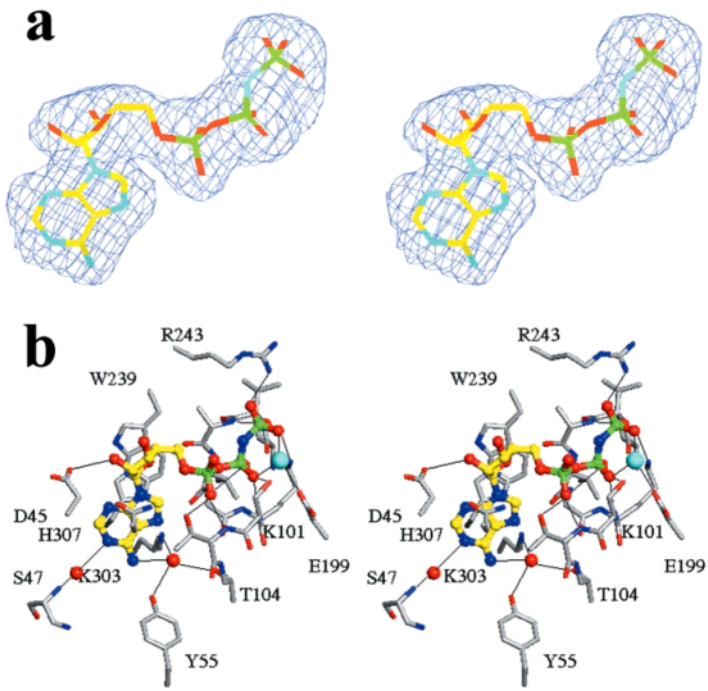


Figure 42 *Stereoview of the overlapping site of two ligands, AMPPNP and CoA. The binding pockets of AMPPNP and CoA are shown in yellow and cyan, respectively. CoA (magenta) and AMPPNP (black) are shown in lines. (a) Residues of the AMPPNP-bound enzyme are shown (in brown) but residues of the CoA-bound enzyme are not shown for clarity. The carboxyl group of Glu249 of the AMPPNP-bound enzyme coincides with the pantetheine moiety of CoA. (b) Residues of the CoA-bound enzyme are shown in green, but residues of the AMPPNP-bound enzyme are not shown for clarity. The carboxyl group of Glu44 of the CoA-bound enzyme coincides with the adenine base of AMPPNP*
(Reproduced with permission from Yuri *et al.*[110])

needs of those studying newly-discovered enzymes. Several citations located in other parts of this chapter can be consulted to give appropriate guidance on methods that employ state-of-the-art laboratory instrumentation. Most of the papers selected for inclusion in this section deal with unusual, often groundbreaking, studies.

8.4.1 Enzyme Activity at Low Temperatures. Enzyme activity at −100 °C has been established for the first time, with beef liver catalase and calf intestine alkaline phosphatase, using either methanol:ethyleneglycol:water (70:10:20) or dimethyl sulfoxide:ethyleneglycol:water (60:20:20) as medium. The implication drawn,[134] that molecular motion is not essential for enzyme activity since the temperature in these experiments is below the dynamic transition state for these proteins, seems to sweep away a range of current assumptions underpinning mechanisms for enzyme catalysis, and will need further experimental support from a broader range of examples.

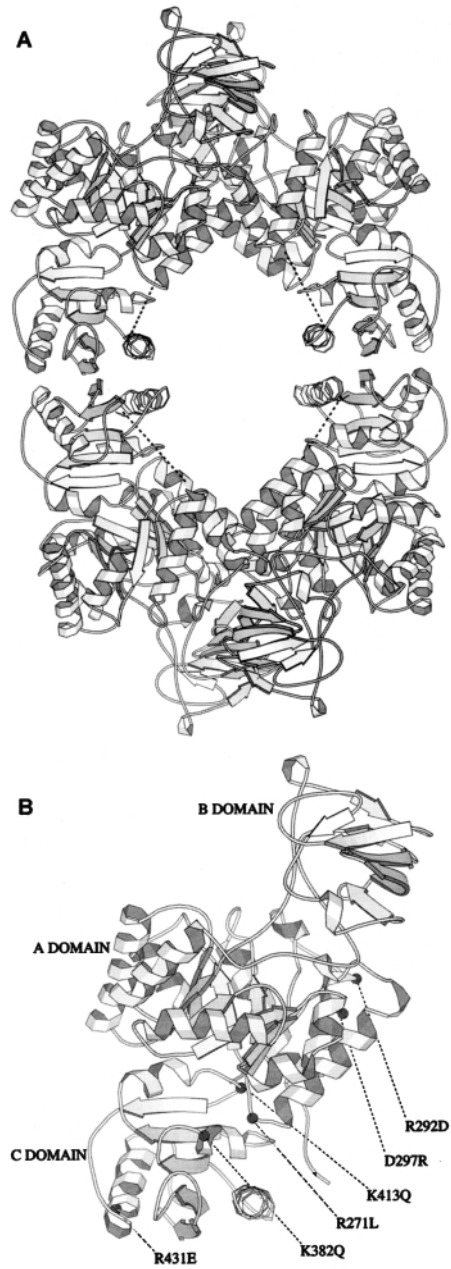


Figure 43 *(A) Schematic representation of the* E. coli *PK crystallized in the inactive T-state. Subunits related by the molecular twofold axis running in the vertical direction. Dashed lines connect Phe345 to Leu352 (the sequence 346 to 351 is disordered). (B) The* E. coli *subunit [top left hand corner of Figure 43(A)]. The Cα atoms of the mutated residues are outline by grey spheres*
(Reproduced with permission from Valentini *et al.*[111])

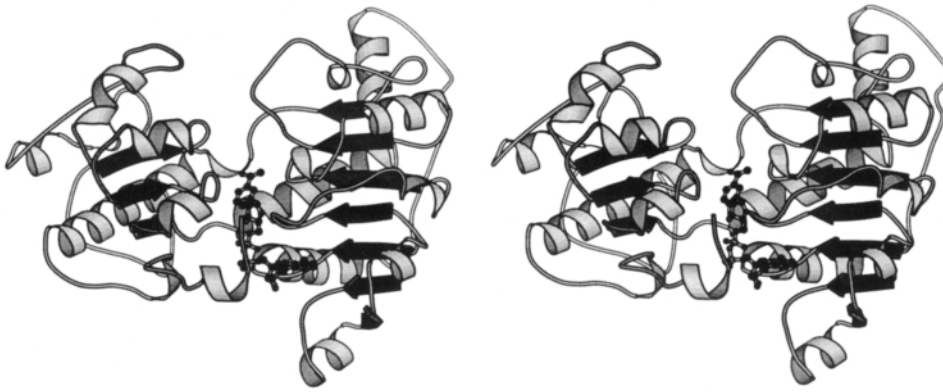


Figure 44 *Subunit of* C. boidinii *FDH model with ATP bound to the active site. The coenzyme binding domain is to the left and the catalytic domain is to the right. NAD^+ is shown as a ball and stick representation*
(Reproduced with permission from Labrou *et al.*,[117] *Eur. J. Biochem.*, Blackwell Science)

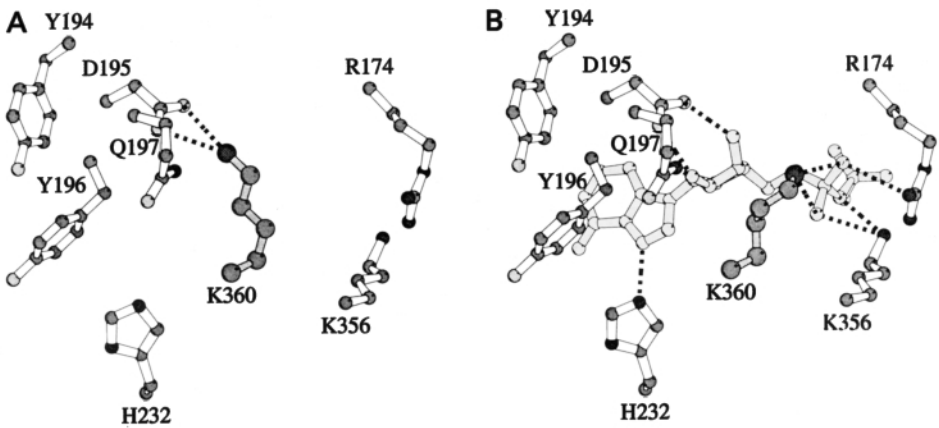


Figure 45 *(A) Diagram showing the side chains of the of* C. boidinii *FDH model that interact with the cofactor NAD^+. The nicotinamide moiety of NAD is omitted for clarity with the remainder of the NAD shown in uniform light grey. Lys360 is shown in dark grey. In the absence of NAD^+, Lys360 forms a salt bridge with Asp195, so explaining its unusually low reactivity to oADP. (B) On binding of NAD^+, a conformation change is suggested to occur, with Lys 360 then becoming a contributor to the interaction with NAD^+*
(Reproduced with permission from Labrou *et al.*,[117] *Eur. J. Biochem.*, Blackwell Science)

8.4.2 Enzyme Activity at Low pH. Aspergillopepsin II, which in spite of its name is a non-pepsin type proteinase, carries aspartate and glutamate residues as essential contributors to the active site, so there is little surprise that the optimum pH for its catalysis of the hydrolysis of milk casein is as low as 2.6 (and the optimum pH for hemoglobin is less than 2).[135] Seven aspartate and five glutamate residues in the enzyme were replaced by site-directed mutagenesis, with aspara-

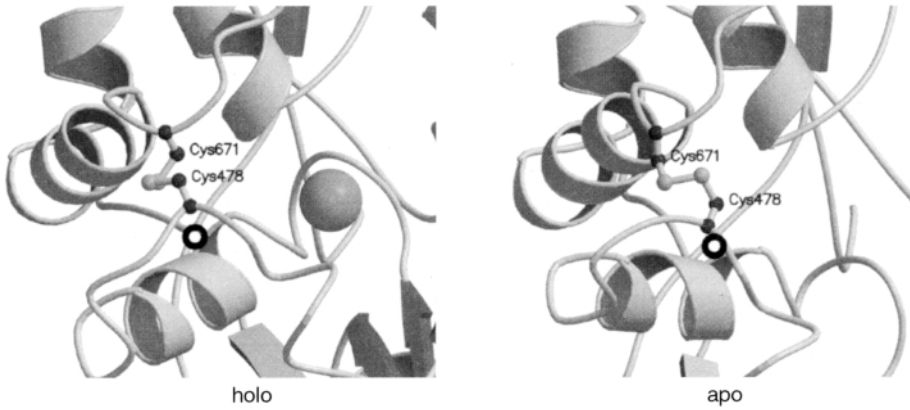


Figure 46 *The Cys478-Cys71 disulfide bond in relation to the screw axis of the C-lobe of apo and holo ovotransferrins. Conformational transitions between the apo and holo forms involve rotation of the two domains about the screw axis that is represented by a circle with a filled circle inside it. The large filled sphere is an iron atom*
(Reproduced with permission from Mauralidhara and Hirose.[118])

gine and glutamine residues, respectively, to pinpoint the active site contributors.

Proton shifts are an inherent part of enzyme catalysis, and have been a constant feature of mechanistic studies. A two-step reaction mechanism (catalysed alternatively by acid and base) with partial proton shuttles and charge redistributions promoted by short strong H bonds (SSHBs) (playing a dual role as an amphi-acid/base catalyst) is proposed to explain the enormous rate enhancement observed in enzymatic reactions involving carbanion intermediates. The SSHBs in the two-step reactions are found to be responsible for enhancing enzyme–substrate interactions in favour of the transition state structure over that of reactant. A study presents evidence of the role of SSHB in driving partial proton shuttles and charge redistributions in ketosteroid isomerase (KSI) for which a large stabilization energy for EIs/TSs relative to ES comes from both the enhanced H-bond energy (of SSHBs relative to normal H bonds) and the MO interaction energy (by the electronic charge redistributions due to charge transfers and polarization involving excess electron dissipation to the catalytic residues and possibly by partial covalent bonding). Thus, the activation barrier is lowered predominantly by both preorganization-driven SSHB and SSHB-driven proton shuttles and charge redistributions. The origin of the catalytic role of SSHB is explained with a dual role of very strong proton donor/acceptor to/from substrate in catalytic activation involved in two-step reactions to be catalysed alternatively by acid and base.

This understanding will help to open up a new avenue for designing novel enzymes and antibodies acting through this mechanism.[136]

8.4.3 Classical Methods of Probing Enzyme Catalytic Mechanisms. The techniques of site-directed mutagenesis (refs. 24, 111, 124, 135), irreversible inhibition (refs. 109, 179), and mass-spectrometric monitoring of reaction mixtures (refs. 23,

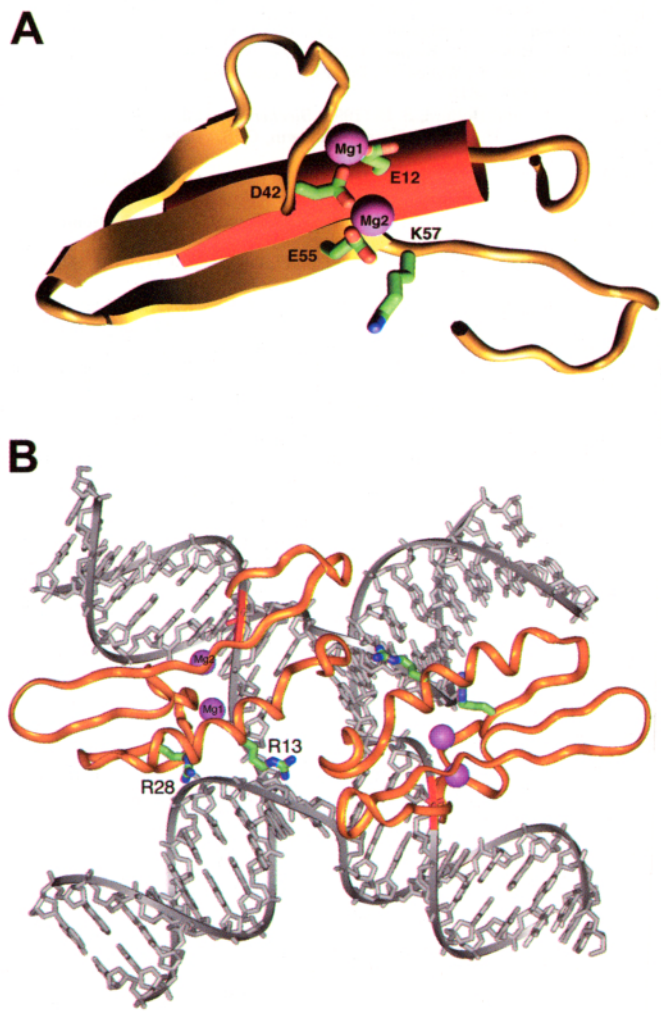


Figure 47 *(A) Model of the catalytic domain of Hjc and its interaction with the Holliday junction (predicted secondary structure). Proposed catalytic residues and metal ions are shown as stick and space filling representations, respectively. (B) The Hjc-junction complex. Catalytic domains are represented by ribbons with the positions of the magnesium ions and the conserved residues Arg13 and Arg28 indicated as stick representations. The model DNA junction structure is shown in grey with a backbone ribbon highlighting the position of junction cleavage in red* (Reproduced with permission from Kvaratskhelia *et al.*[124])

25) are discussed elsewhere in this chapter. Another less targetted way for probing protein structure is illustrated for the pyruvate decarboxylase component of the pyruvate dehydrogenase multi-enzyme complex of *Bacillus stearothermophilus*, with limited trypsin proteolysis being shown to affect the active site.[137]

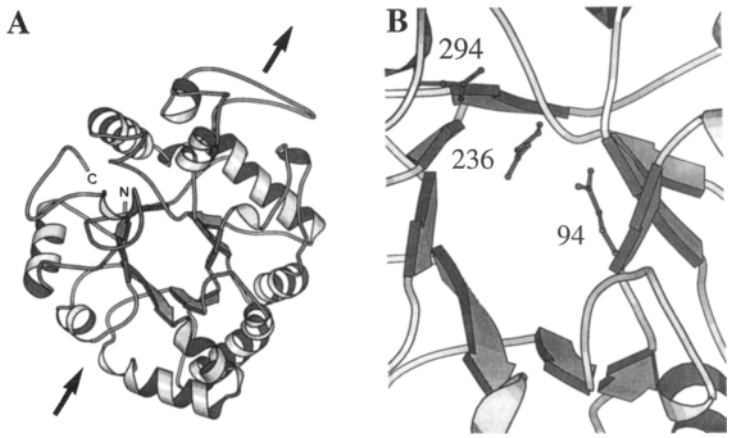


Figure 48 *Banana β-1,3-glucan endohydrolase. (A) β-Sheet strands form a crown surrounded by an outer crown of α-helices to form the $(\beta/\alpha)_8$ TIM-barrel. Arrows indicate the line along which the active site lies within the structure. (B) Localization of three conserved glutamate residues entering the catalytic groove*
(Reproduced with permission from Peumans *et al.*,[131] *Eur. J. Biochem.*, Blackwell Science)

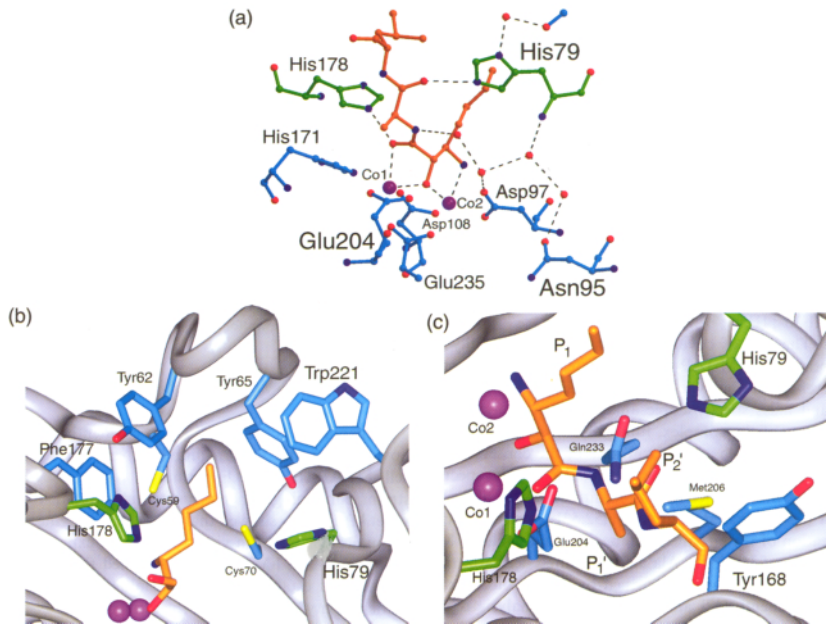


Figure 49 *Active site interactions of methionine aminopeptidase with a bestatin-type inhibitor. His79 and His178 are important contributors to the mechanism of action of the enzyme. Two cobalt ions are shown as spheres near the inhibitor, depicted in (a–c) as a skeleton structure*
(Reproduced with permission from Lowther and Matthews[132] and Elsevier Science)

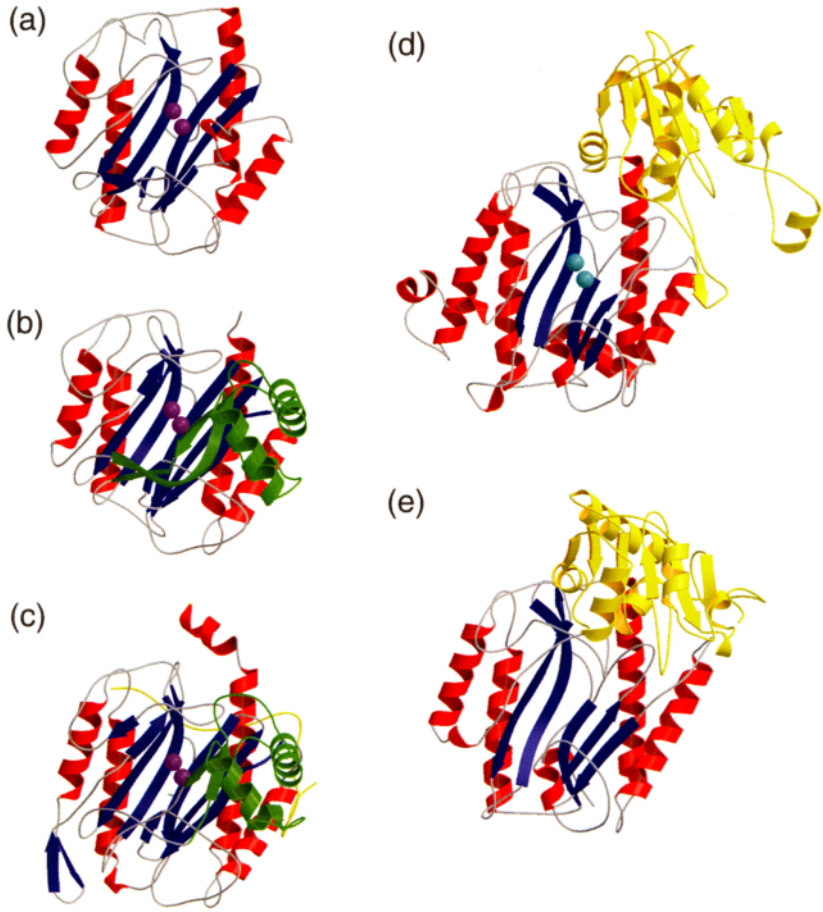


Figure 50 *Structures (a–e) are the known type I and type II methionine aminopeptidases characterised by β-sheet arrangements that lead to the whimsical name 'pitta bread' enzymes. Cobalt or manganese ions are held within these structures (see Figure 49)*
(Reproduced with permission from Lowther and Matthews[132] and Elsevier Science)

The mechanism of action of 8-(*S*)-amino-7-oxononanoate synthase has been elucidated based on spectroscopic, kinetic and crystallographic studies.[138] This is a pyridoxal-5′-phosphate dependent enzyme that catalyses the decarboxylative condensation of L-alanine with pimeloyl-coenzyme A in a stereospecific manner (Scheme 3).

8.4.4 Intra-enzyme Contacts Established by Photo-crosslinking. Azidophenylacetylation of a cysteine residue followed by photo-crosslinking has been used to probe contacts between the ribonuclease H domain of HIV-1 reverse transcriptase (Figure 52).[139]

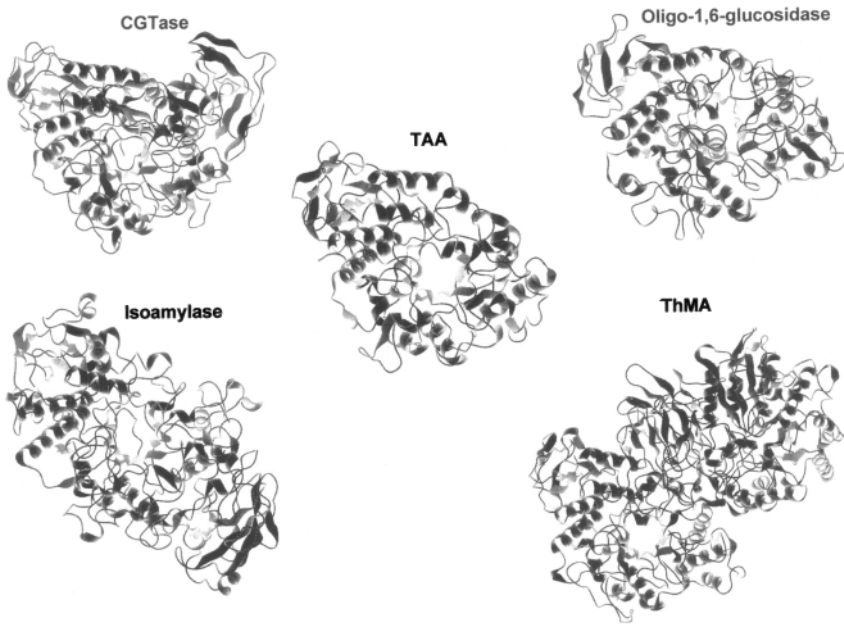


Figure 51 *Glycoside hydrolase Family 13 members [CGTase* = Bacillus circulans; *oligo-1,6-glucosidase* = Bacillus cereus; *TAA* = *α-amylase of* A. oryzae; *isoamylase* = Pseudomonas amyloderamosa; *ThMA* = *CD-degrading enzyme from* Thermus *sp*]
(Reproduced with permission from Park *et al.*[133] and Elsevier Science)

Peroxynitrite (a mixture of NO and the superoxide anion O^{2-}) inactivates cytochrome P_{450}BM3. The essential groups that are targetted in this process are considered to be tyrosine residues which undergo nitration and thiol groups that are oxidized by ferryl species.[140]

A tyrosine residue activates the epoxide moiety of a substrate to attack by an aspartate carboxylate ion (Scheme 4) in the catalytic cycle of the widely distributed enzyme epoxide hydrolase, on the way to the product (a 1,2-diol).[141]

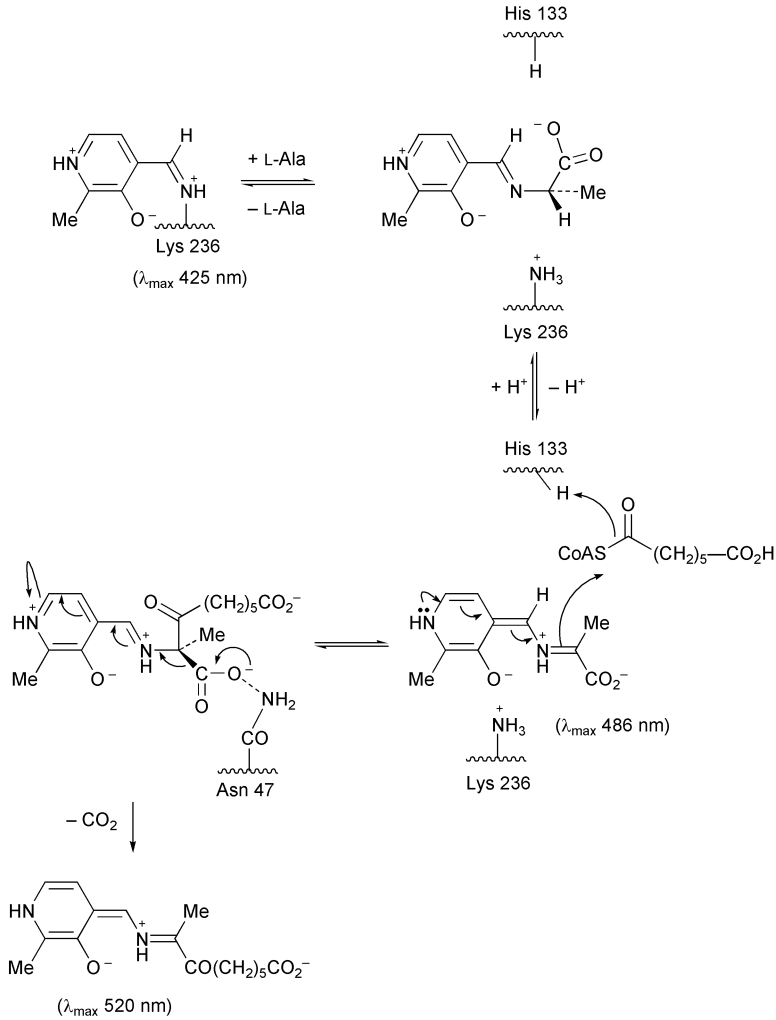
An endonuclease of the amoeboflagellate *Naegleria* has a zinc ion skirted by cysteine and histidine residues as part of the active site (Scheme 5).[142]

Hydrolysis of ATP by β-actin (see also ref. 48) involves arginine residue 177 as a constituent of the active site (Figure 53).[143]

A pancreas-specific glycosylated protein disulfide isomerase binds substrate peptides through their tyrosine and tryptophan residues.[144]

A protein required for disulfide bond formation *in vivo* uses quinones (which show a propensity to act as electron aceptors) as redox reagents in a novel catalytic process.[145]

The nonapeptide sequence -Arg-Trp-Thr-Asn-Asn-Phe-Arg-Glu-Tyr- (residues 183–191) of matrix metalloproteinase MMP-1 is critical for the expression of collagenolytic activity of this enzyme;[146] this sequence makes up the catalytic domain (Figure 54), in combination with the C-terminal hemopexin domain and other structures, to fulfil a role in the cleavage of the collagen triple helix.



Scheme 3 *8-(S)-Amino-7-oxononanoate synthase mechanism deduced from spectroscopic assignments*
(After Webster *et al.*[138])

Based on UV-visible spectroscopic data, Baldwin's group has concluded that a $[2Fe–2S]^{2+}$ cluster characteristic of wild-type biotin synthase is also a feature of mutants prepared from it by replacing each of the three conserved cysteine residues with an alanyl residue;[147] Baxter's group has provided complementary results for [Fe–S] cluster-binding residues of *E. coli* biotin synthase, and a conclusion that the conserved cysteine residues Cys53, Cys57 and Cys60, are crucial for [Fe–S] cluster-binding while Cys188 plays a different, unknown, structural role.

The arachidonic acid component of a complex with murine apo-cyclo-oxygenase and prostaglandin possesses a distorted conformation (X-ray

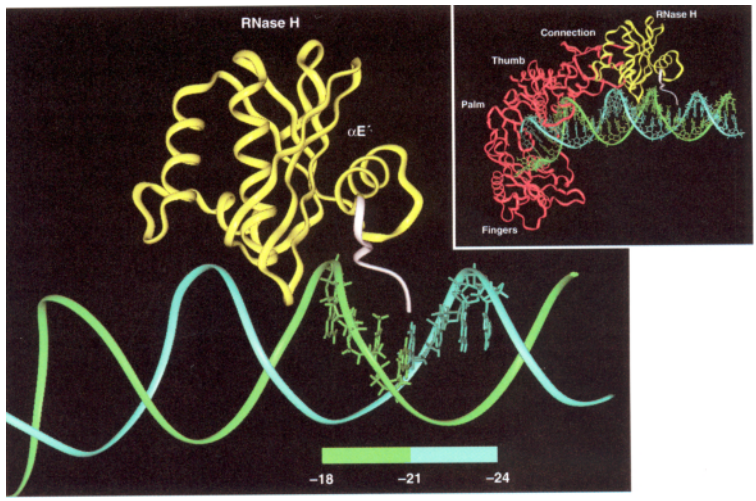
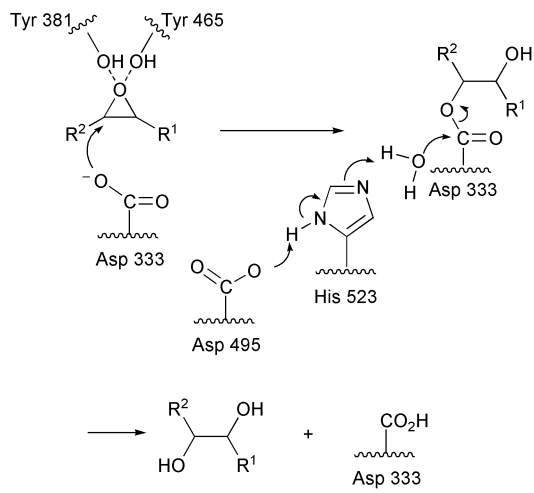
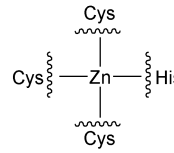


Figure 52 *Positioning of the RNase H domain within duplex DNA of HIV-1 RT to account for azidophenacyl-mediated cross-linking to template and primer nucleotides. All structural features of HIV-1 RT other than the C-terminal p66 RNase H domain have been omitted for clarity. Within the RNase H domain, the last structural element, α-helix E′, has been indicated for reference. The white portion represents C-terminal RNase H residues. Native residues 559 and 560 along with the cysteine introduced at position 561 modelled onto the published X-ray structure are shown in white. Template and primer nucleotides are green and blue, respectively, as are the nucleotides to which azidophenyl-mediated cross-linking could be achieved*
(Reproduced with permission from Rausch *et al.*[139])



Scheme 4 *Mechanism of oxirane cleavage catalyzed by epoxide hydrolase*
(After Yamada *et al.*[141])



Scheme 5 *Active site detail of endonuclease from* Naegleria (After Elde *et al.*[142])

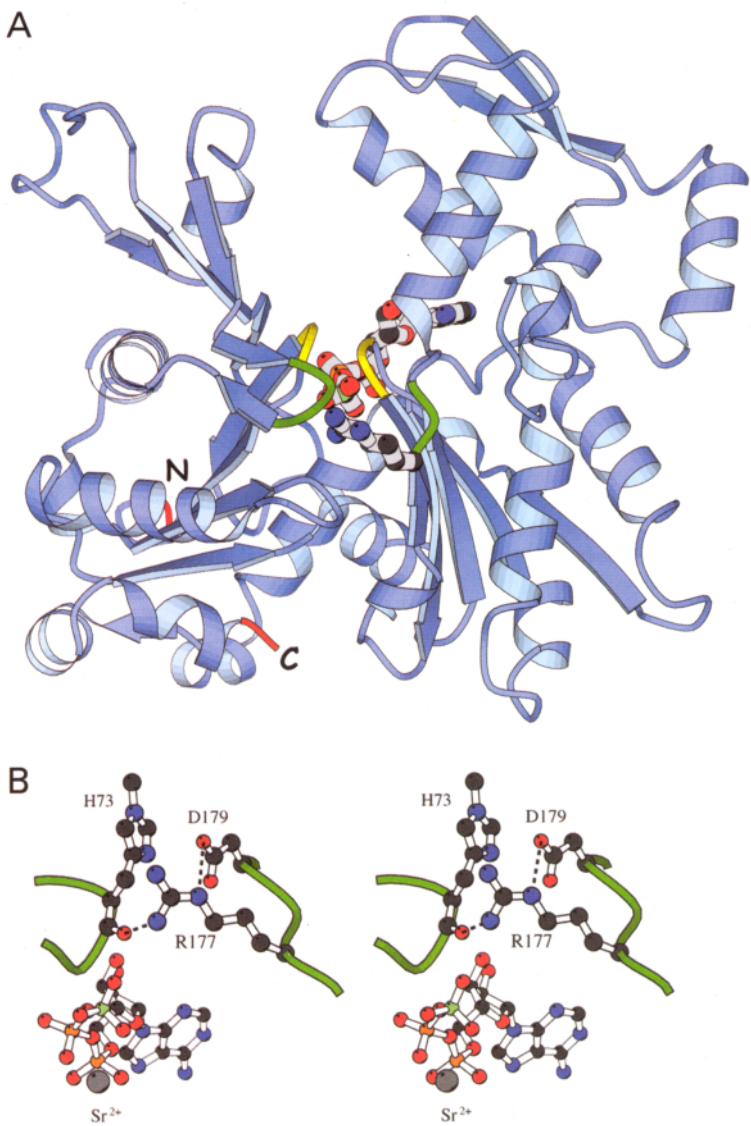


Figure 53 *(A) Overview of β-actin (see also Figure 6). The bound nucleotide+cation complex and the side chain of Arg177 are shown. (B) Close-up view around Arg177*
(Reproduced with permission from Schuler *et al.*,[143] *Eur. J. Biochem.*, Blackwell Science)

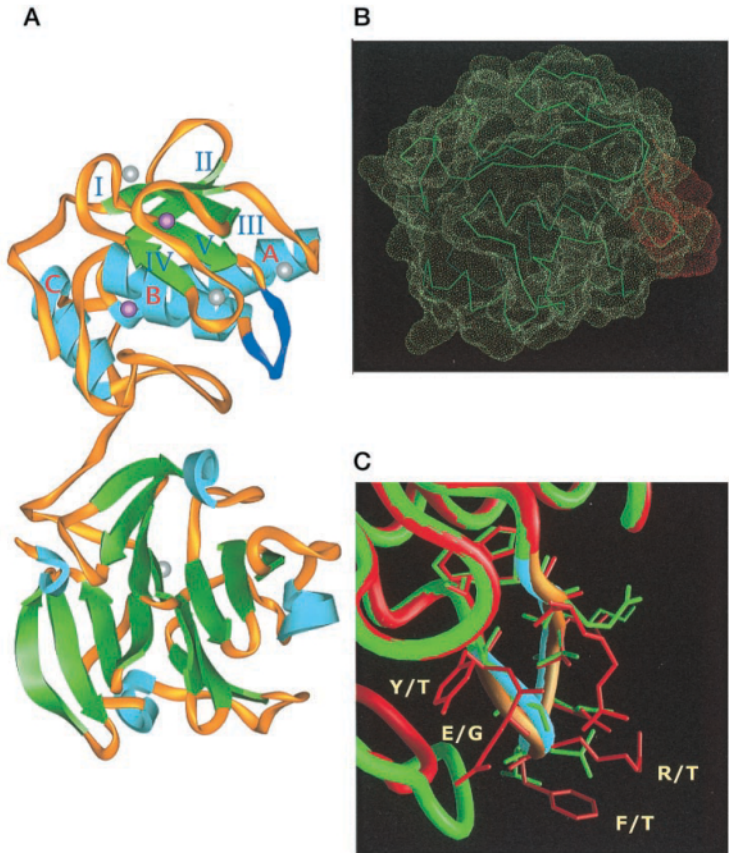


Figure 54 *Nonapeptide and matrix metalloproteinase-1 (MMP-1) and analogue MMP-3 (stromelysin-1 into which various lengths of MMP-1 were introduced), mentioned in the text. Light blue for α-helices, green for β-strands, gold for connecting loops; Zn and Ca ions are purple and grey spheres, respectively; the nonapeptide 183-191 is dark blue. (A) Ribbon diagram (upper and lower domains are the catalytic and hemeopexin domains, respectively). (B) Surface of catalytic domain of MMP-3. Surface of 183-191 (red) superimposed on the MMP-3 catalytic domain (green). (C) Superimposition of MMP-1 (red) and MMP-3 (green) on the nonapeptide*
(Reproduced with permission from Chung *et al.*[146])

crystal structure analysis) that is suggestive of an important role for this component, in setting up this enzyme that starts off eicosanoid synthesis *in vivo*.[148]

8.5 Heme-binding Enzymes. – Heme-binding aspartic proteinase from eggs of the hard tick (*Boophilus microphus*) must possess a docking site for heme so as to explain the increased specificity of the enzyme towards hemeproteins, and thereby suggesting a novel way of regulating the activity of proteinases.[149]

8.6 Proenzymes. – A glycoprotein α-subunit with 92 amino acid residues including five disulfide bonds has been found to combine with four distinct

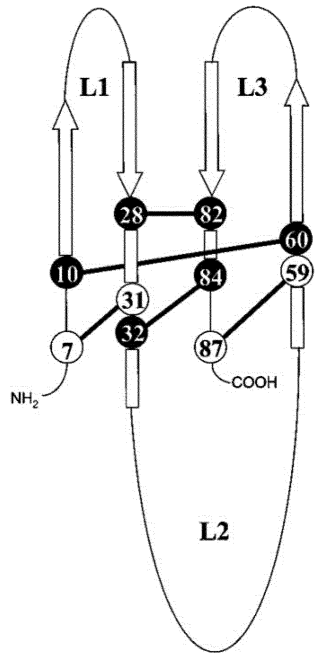


Figure 55 *The glycoprotein α-subunit featured in this study*
(Reproduced with permission from Darling *et al.*[150])

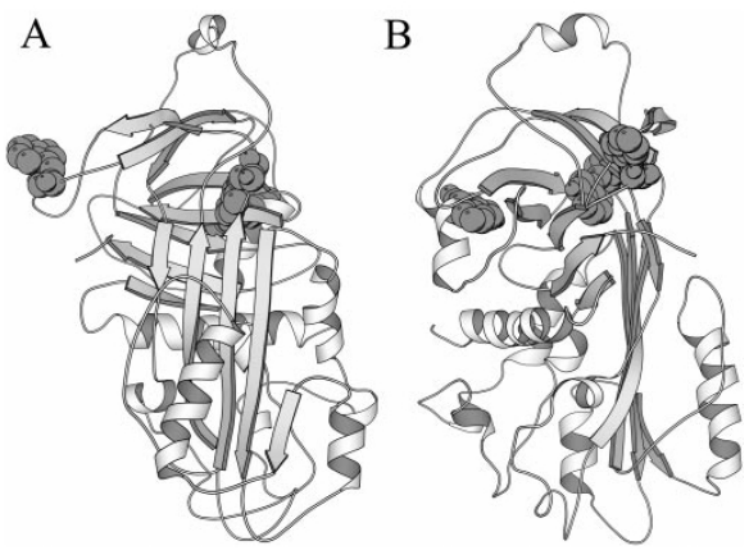


Figure 56 *(A) Schematic diagram of native α-antichymotrypsin (ACT). (A) Front view of ACT, with Trp194, Trp215 and Trp276 shown as van der Waals spheres. (B) Side view, highlighting the location of Trp276*
(Reproduced with permission from Pearce *et al.*[152])

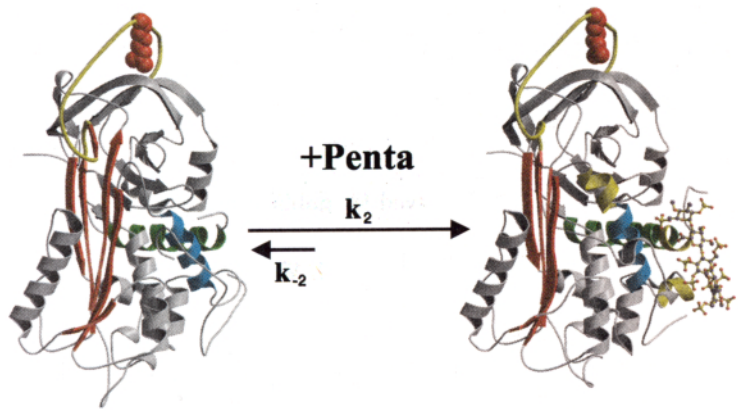


Figure 57 *Native and pentasaccharide-bound antithrombins indicating the structural changes in secondary structure that accompany binding. The pentasaccharide is shown in ball and stick form. The central β-sheet (red), reactive centre loop (yellow), helix D (cyan), helix A (green), and P1 arginine (red) are shown; secondary structural changes in the pentasaccharide-bound structure are yellow* (Reproduced with permission from Huntington *et al.*[155])

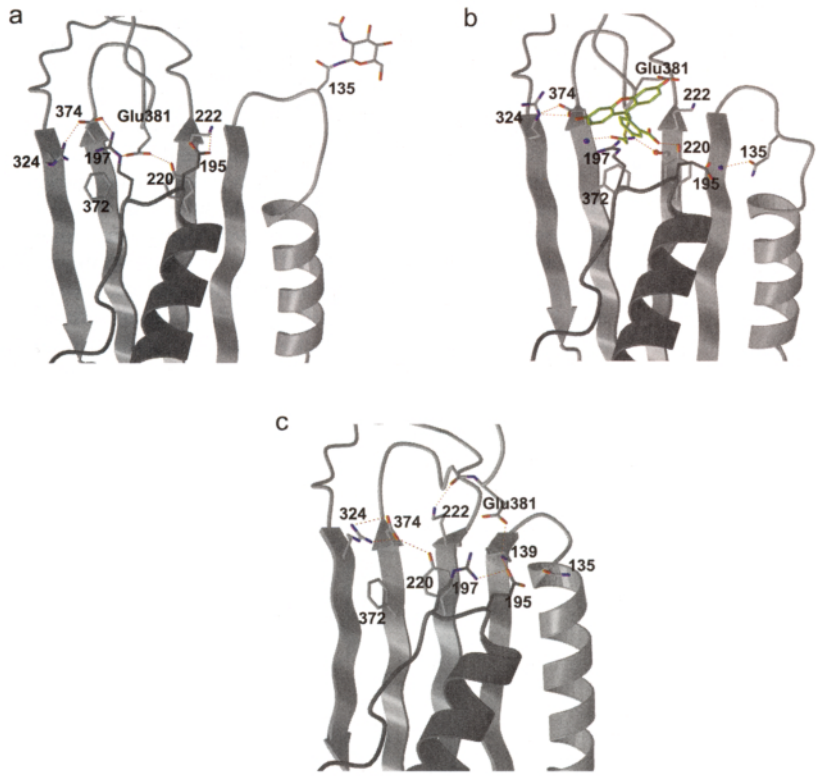


Figure 58 *Hinge region interactions for native (a) P14-fluorescein (b) and pentasaccharide-bound (c) antithrombins*
(Reproduced with permission from Huntington *et al.*[155])

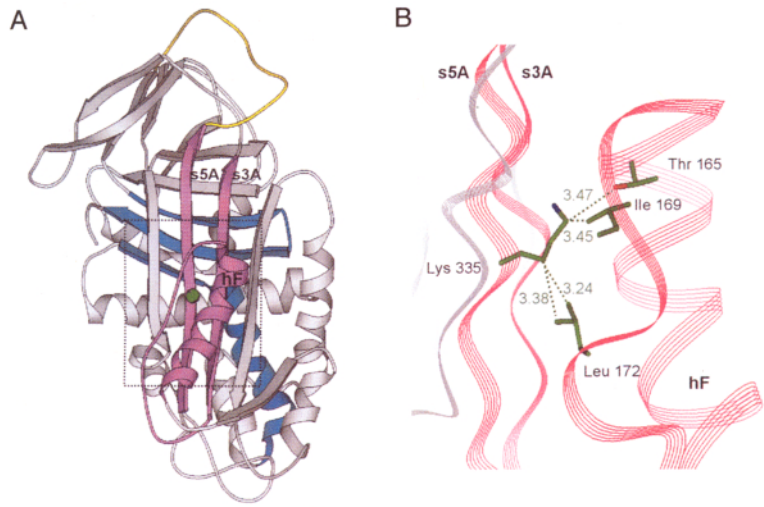


Figure 59 *Native α-antithrombin (see also Figure 57). (A) This structure includes a rectangle containing Lys335 and the contents of this rectangle are re-drawn in (B) showing the side chains of Lys335 and of neighbouring residues*
(Reproduced with permission from Im and Yu.[156])

β-subunits for the genesis of four hormones: chorionic gonadotrophin, luteinizing hormone, thyroid-stimulating hormone and follicle-stimulating hormone. Conventional residue replacement (Cys replaced by Ala) in a planned fashion shows the importance of some of the disulfides comprising the cystine knot (Figure 55) in sustaining a suitable three-dimensional structure for the system.[150]

8.7 Proteins as Enzyme Inhibitors. – A preceding section covering binding and adhesion involving proteins could also have been used to cover proteins as enzyme inhibitors, since binding ability is inherent in such behaviour. Plasma hyaluronan-binding protein shows the properties of a serine protease that can be inhibited by several proteins, including the Kunitz-type protease inhibitor domain of amyloid β-protein precursor.[151] Another Kunitz type protease inhibitor is referred to in ref. 169.

Serpins fulfil essential regulatory roles, but trauma leading to their misfolding and aggregation can lead to breakdown or imbalance of systems in which they are involved. This is implicated in a number of diseases, ranging from emphysema and liver disease, to cancer and dementia, for example, so considerable effort is being devoted to the folding pathways in this area, and stable intermediates I_1 and I_2 in the folding pathway (N to I_1 to I_2 to U, and the reverse pathway; N = native, U = unordered) of α_1-antichymotrypsin have been established to have lost 20% of the secondary structure of the native conformation N (Figure 56).[152]

This serpin, like α_1-proteinase inhibitor and α_1-macroglobulin, prevents adhesion molecules such as fibronectin from undergoing degradation and they are thus retained in an available state.[153] The conformational stability of the serpin

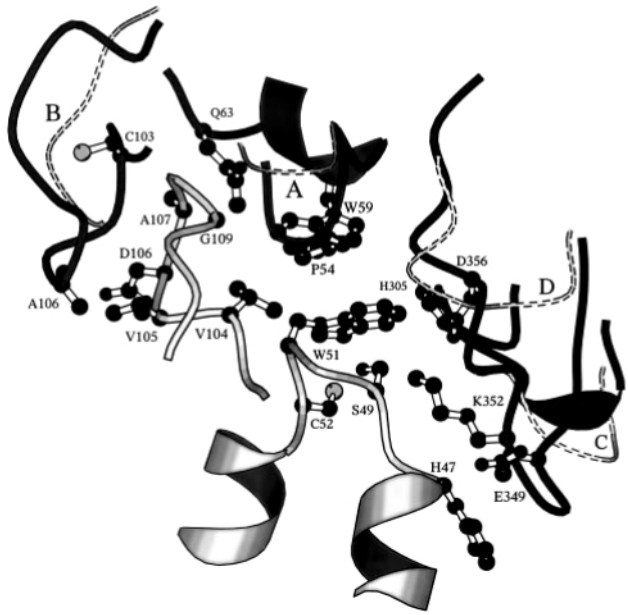


Figure 60 *Selected interactions between amylase (main chain shown in grey) and inhibitor (main chain shown in white) in the HAS-0.19 complex. Thinner dotted lines are shorter enzyme loops, labelled A–D. In the TMA complex His305 is partly obscured by Asp356 and Trp59 is partly hidden by Pro54*
(Reproduced with permission from Franco *et al.*,[159] *Eur. J. Biochem.*, Blackwell Science)

α_1-antitrypsin is increased in citrate-containing media.[154]
Antithrombin is a serpin with the unique property of circulating in an inactive native conformation, activation requiring binding to a specific pentasaccharide sequence (Figures 57, 58) that is found in heparin.[155]

α_1-Antitrypsin and other serine protease inhibitors are proteins that are in a metastable state in their native form (Figure 59).[156] Lys-335 is critical for controlling the activity of the inhibitor.

An X-ray structure for this serpin has been published.[157] A 30-residue extension loop allows conversion of the structure into a very stable state.[158]

X-Ray structures of human and insect salivary α-amylases complexed with inhibitors have provided improved understanding of these enzymes and may assist the rational design of new and simpler inhibitors (Figure 60).[159]

Natural inhibitors of proteinases are very rarely encountered, but a novel inhibitor, *Streptomyces caespitosus* neutral protease inhibitor, of the metalloprotease from *Streptomyces caespitosus* has been isolated from *Streptomyces* sp. I-355.[160] It also inhibits the serine proteinase subtilisin, so conforming to the general behaviour of several other natural proteins, but as a 'double headed' inhibitor it stands apart.

Clitocypin is a novel cysteine proteinase inhibitor from *Clitocybe nebularis*.[161]

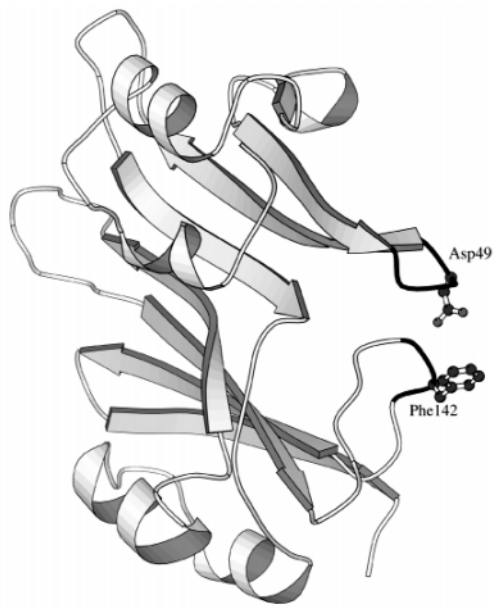


Figure 61 *The location of randomized residues in β-lactamase inhibitory protein (BLIP) around Asp49 and Phe142 featured in this study are shown in black*
(Reproduced with permission from Huang *et al.*[166])

Prodomains of several cysteine proteases of the papain family are potent inhibitors of their parent enzymes, cathepsins K, L and S.[162] A 152-residue protein from kiwi fruit (*Actinidia chinensis*) acts as a powerful inhibitor of plant pectin methylesterase; this adds to a growing list of proteins that inhibit enzymes involved in sugar metabolism.[163]

A new β-lactamase inhibitory protein has been located in *Streptomyces exfoliatus* SMF19.[164]

'Family 12' proteins of *Helicobacter pylori* are β-lactamases of a new class, designated class E.[165] A β-lactamase inhibitory protein (Figure 61) binds tightly to several members of this broad class of enzymes, and is a promising candidate for pharmaceutical development since the binding affinity of this protein can be adjusted by protein engineering.[166]

Other studies in the same vein include *Pseudomonas aeruginosa* β-lactamase PSE-4.[167]

Two ribosome-inactivating proteins have been isolated from Solomon's seal (*Polygonatum multiflorum*).[168]

The hookworm *Ancylostoma ceylanicum* secretes a broad spectrum Kunitz-type serine protease inhibitor (see also ref. 151).[169] Scorpine, a novel 75-residue protein from the scorpion (*Pandinus imperator*) with three disulfide bridges and a unique sequence for an insect protein, has antibacterial properties and is a potent inhibitor of ookinete and gamete stages in the life cycle of *Plasmodium berghei*.[170]

Self-inhibition illustrated by myosin light chain kinase may be just one

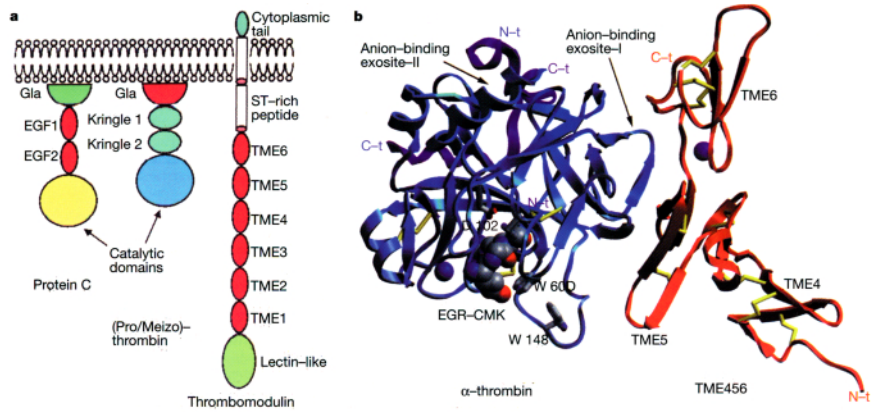


Figure 62 *The protein C anticoagulant pathway. (a) Domain organisation of protein C. (b) Complex between α-thrombin and TME456, alkylated with the inhibitor, L-Glu-Gly-L-Arg-chloromethyl ketone (the alkyl group introduced using this reagent is shown in ball form in α-thrombin). A sodium atom associated with TME456 is shown as a sphere*
(Reproduced with permission from Fuentes-Prior *et al.*[179] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

example of a more extensive phenomenon. The enzyme contains a regulatory segment, an autoinhibitory region and a calmodulin-binding sequence that can fold back on its catalytic core to inhibit kinase activity.[171]

8.8 Enzyme Inhibition by Non-protein Species. – These studies continue to offer valuable insights into enzyme behaviour that often give useful pointers to clinical applications using effective inhibitors as medicaments.

The first evidence has been acquired that all-*trans* retinoic acid interacts with protein kinase C, one of a family of key regulatory enzymes in signal transduction. This is important since it effectively decreases the activity of the enzyme and may have implications for cancer therapy.[172]

An ongoing topic of pharmaceutical interest concerns the retroviral proteinase that is responsible for the maturation of the HIV nascent viral particle to an infectious entity. Thorough reviews are available, covering the results of all published studies.[173]

Inhibitors of one of the most intensively studied enzyme families, the caspases (see also refs. 109, 126, 174, 176), offer rewards in terms of novel treatment of diseases characterized by excessive apoptosis, such as osteoarthritis.[174] Benefits are also likely to follow, from successful intervention into the action of caspases that are linked to Alzheimer's disease,[175] with caspase-12 now known to have direct links with the disease.[176]

A compendium of theories for the cause of Alzheimer's disease occupies a volume of *Annals of the New York Academy of Sciences*.[177]

The sequence of events in blood protein coagulation is also an ongoing topic of investigation with more refinement of the research objectives being shown. Peptides are now seen to bind at exosites rather than the catalytic sites of

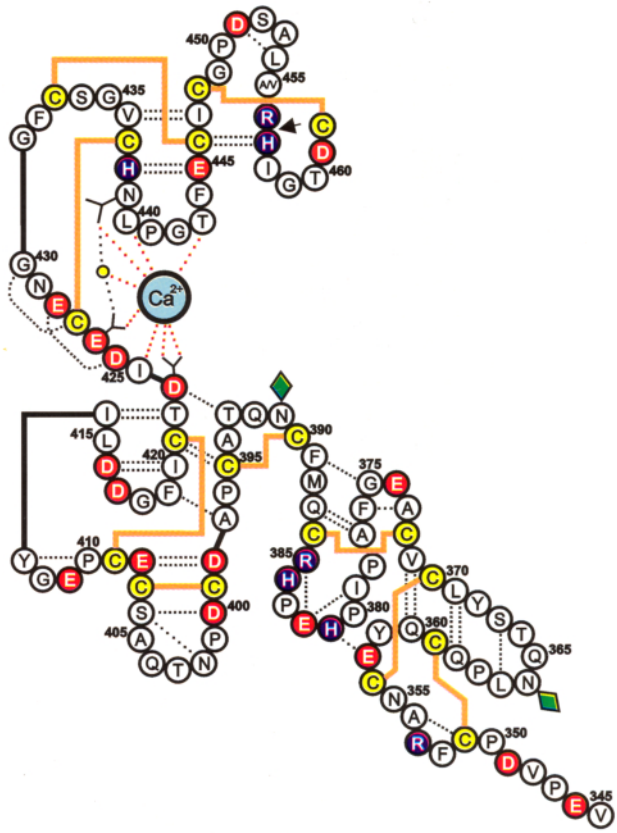
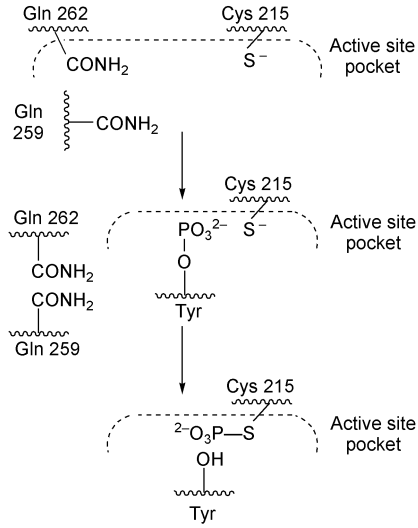


Figure 63 *Sequence of human TME456 fragment (some secondary structure features are indicated)*
(Reproduced with permission from Fuentes-Prior *et al.*[179] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

thrombin and related proteins that possess serine proteinase activity. The result is to block the activation of the tissue factor–factor VIIa complex in the fibrin clot-formation process, and so peptides offer themselves as potential anti-coagulants.[178] Thrombin irreversibly inhibited by reaction with L-Glu-Gly-L-Arg-chloromethyl ketone has been combined with thrombomodulin, for assessment of the anti-coagulant activity of the complex (Figures 62, 63).[179]

9 Signal Regulatory Proteins

9.1 Receptor-like Membrane Proteins. – Membrane proteins are the most biomedically important family of proteins, according to ref. 180, a statement that seems exaggerated to a reader thinking over a long list of other physiologically important proteins. The claim is made on the basis that the majority of pharma-



Scheme 6 *Energy penalty arising from Gln259-Gln262 interactions in or near the active site of protein tyrosine phosphatase-α. The alternative scheme involving Gln262 outside the active site involves no energy penalty*
(After Peters *et al.*[182])

ceutical agents are directed at membrane protein targets. The application of standard methods of structure determination to membrane proteins is very difficult, and less than 0.2% of the Protein Data Bank comprises proteins in this category.[180]

Receptor-like trans-membrane proteins contain a cytoplasmic proline-rich region and four cytoplasmic tyrosine residues that, when phosphorylated, bind SH2 domain containing tyrosine phosphatases. One of these enzymes, SIRPα1,[181] has been suggested to be a negative regulator of growth hormone signalling. Residue 259 (a glutaminyl residue) is a major determinant of substrate specificity of protein tyrosine phosphatases 1B and α (Scheme 6).[182]

Anchorage on a membrane by a 20-residue peptide (an analogue of the fusion peptide of the influenza virus hemagglutinin) has been shown to depend on pH.[183] Two of the five glutamate residues of the peptide were concluded to be crucial to the anchorage.

9.1.1 Presenelins. Presenelins 1 and 2 are polytopic membrane proteins that are mutated into β-amyloid proteins in most of the early onset Alzheimer's disease cases, but the non-conserved hydrophilic loop domain is not essential to this behaviour.[184] These mutations are followed by perturbed Ca^{2+} homeostasis; this was something of a mystery until the binding of sorcin by presenelins was appreciated, the sorcins being well-known to be involved in Ca^{2+} channel modulation.[185]

It has become apparent in the last few years that a compelling strategy to treat and prevent Alzheimer's disease may involve understanding first how certain integral membrane proteins undergo unusual proteolytic cleavages within their

transmembrane domains, and then to work on inhibiting this process pharmacologically. The substrate of interest in Alzheimer's disease is the amyloid precursor protein, but several other proteins apparently are cleaved by a highly similar or identical proteolytic activity within their respective transmembrane domains, including the Notch family of cell-surface receptors required for cell fate determination and the Ire1 proteins that initiate signalling in the unfolded protein response pathway. According to ref. 186, the role of secretase is to cleave PS within a membrane domain (see also refs. 187 and 188 for a 'commentary' on this topic).

An unusual intramembranous cleavage of the β-amyloid precursor protein by γ-secretase is the final step in the generation of amyloid β-peptide (Aβ). Two conserved aspartates in transmembrane (TM) domains 6 and 7 of presenilin (PS) 1 are required for Aβ production by γ-secretase. The C-terminal fragments, C83 and C99, of β-amyloid precursor protein are the direct substrates of γ-secretase and can be co-immunoprecipitated with both PS1 and PS2. PS/C83 complexes were detected in cells expressing endogenous levels of PS. These complexes accumulate when γ-secretase is inactivated either pharmacologically or by mutating the PS aspartates. PS1/C83 and PS1/C99 complexes were detected in Golgi-rich and trans-Golgi network-rich vesicle fractions. In contrast, complexes of PS1 with APP holoprotein, which is not the immediate substrate of γ-secretase, occurred earlier in endoplasmic reticulum-rich vesicles. The major portion of intracellular Aβ at steady state was found in the same Golgi/trans-Golgi network-rich vesicles, and Aβ levels in these fractions were markedly reduced when either PS1 TM aspartate was mutated to alanine. Furthermore, *de novo* generation of Aβ in a cell-free microsomal reaction occurred specifically in these same vesicle fractions and was markedly inhibited by mutating either TM aspartate. Thus, PSs are complexed with the γ-secretase substrates C83 and C99 in the subcellular locations where Aβ is generated, indicating that PSs are directly involved in the pathogenically critical intramembranous proteolysis of APP.

Mutations in the presenilin-1 and -2 genes account for about 50% of early onset familial Alzheimer's disease cases. Proteolytic cleavages of the integral membrane protein, β-amyloid precursor protein (APP), result in generation of the 40- and 42-residue amyloid β-peptides that accumulate to high levels in brain regions important for memory and cognition in Alzheimer's disease. APP is cleaved by β-secretase to generate a 99-residue C-terminal fragment that then is cleaved by γ-secretase to generate Aβ40 and Aβ42. γ-Secretase has a critical role in determining the amount of Aβ produced by cells, but its identity is not definitively established.[189]

A model for the neuronal Rab-Sec1-syntaxin 1a system in relation to membrane fusion has been investigated by X-ray crystal analysis (Figure 64).[190]

From previous studies, the presence of a parallel four-helix bundle at the membrane is thought to be a contributory factor for ensuring membrane fusion.

An extraordinary series of deductions has led to elucidation of the pathway followed during the passage of a proton across the cell membrane in the photocycle of bacteriorhodopsin (Figure 65). The detail that has been drawn out

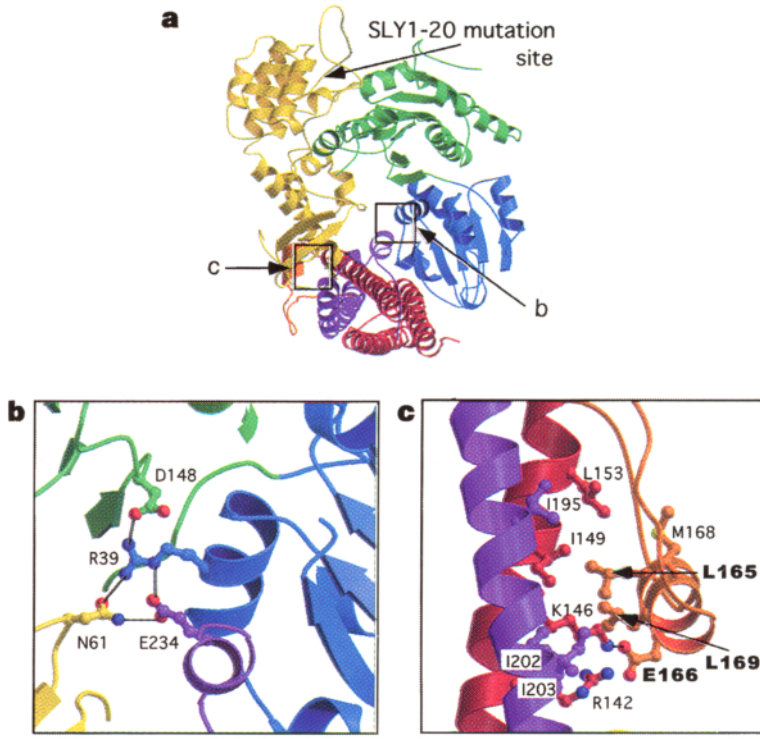


Figure 64 *(a) Yeast SLY1-20 mutant on the nSec1-syntaxin 1a complex. (b) View of the Rop Arg50 to Cys50 mutation position. (c) View of the syntaxin 1a Glu165 to Ala/Leu166 to Ala mutations in the Habc/H3 linker region. Hydrophobic residues that face the linker from H3 and Hc are shown in (c)*
(Reproduced with permission from Misura *et al.*[190] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

includes movements of neighbouring helices induced by the retinal Schiff base that are an essential feature for this process.[191]

10 Processing of Proteins Relevant to Their *In Vivo* Functions

Maillard reactions, classically the preserve of amino acid chemistry, have been recognized as applicable to protein substrates under attack *in vivo* from advanced glycation end-products. Crosslinks in β-amyloid proteins are introduced in this way, and resulting insoluble deposits are the hallmark of the onset of Alzheimer's disease. Significantly, glycation is accelerated by metal ions (Cu^{+}, Cu^{2+}, Fe^{2+}, Fe^{3+}),[192] but little of value can be extracted from this observation, in terms of preventative therapy.

10.1 Domains of Prion Proteins. – The prion domain (Ure2p; the first 65 amino acid residues of the N-terminus) of yeast (*Saccharomyces cerevisiae*) induces autocatalytic formation of amyloid fibres by a recombinant fusion protein. This

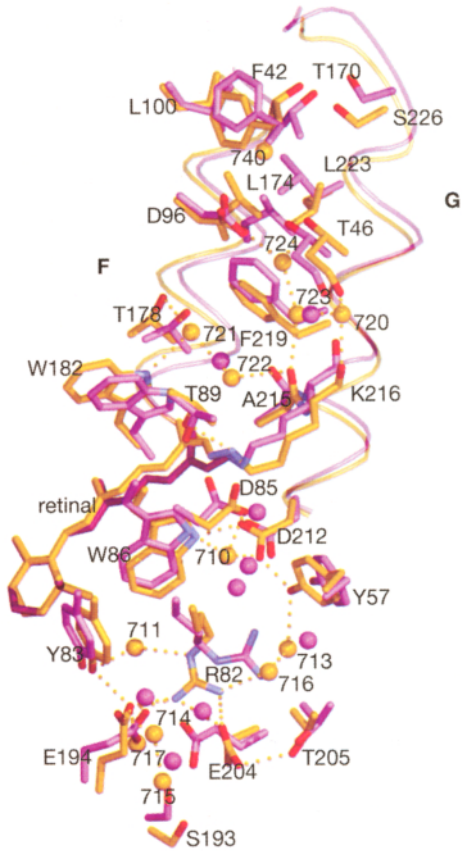


Figure 65 *The proton pathway through which bacteriorhodopsin deals with retinal photochemistry. Bacteriorhodopsin is shown in purple, superimposed by its structure at a late stage in the process shown in yellow. This figure shows movements of regions of the protein that accompany light absorption. Retinal bound as a Schiff base at Lys216 and the Asp85 residue (labelled D85) are shown. This aspartate residue is the first proton recipient from the Schiff base*
(Reproduced with permission from Sass et al.[191] and *Nature*, copyright 2000, Macmillan Magazines Ltd)

demonstrates the ability of this domain to mediate a prion-like conversion process among a broader range of proteins.[193]

Membrane protein *O*-mannosylation at the endoplasmic reticulum by dolichyl phosphate with mannose:protein-*O*-mannosyl transferases, a process of fundamental importance in numerous physiological processes, is the subject of a detailed study.[194] A number of membrane insertion points were identified (Figure 66).

10.2 Inteins and Exteins. – The first example of a true precursor to an intein arises for VMA29 as the mini-precursor of PI-SceI; a crystal structure gives a view of the pre-spliced state of the precursor (Figures 67–69).[195]

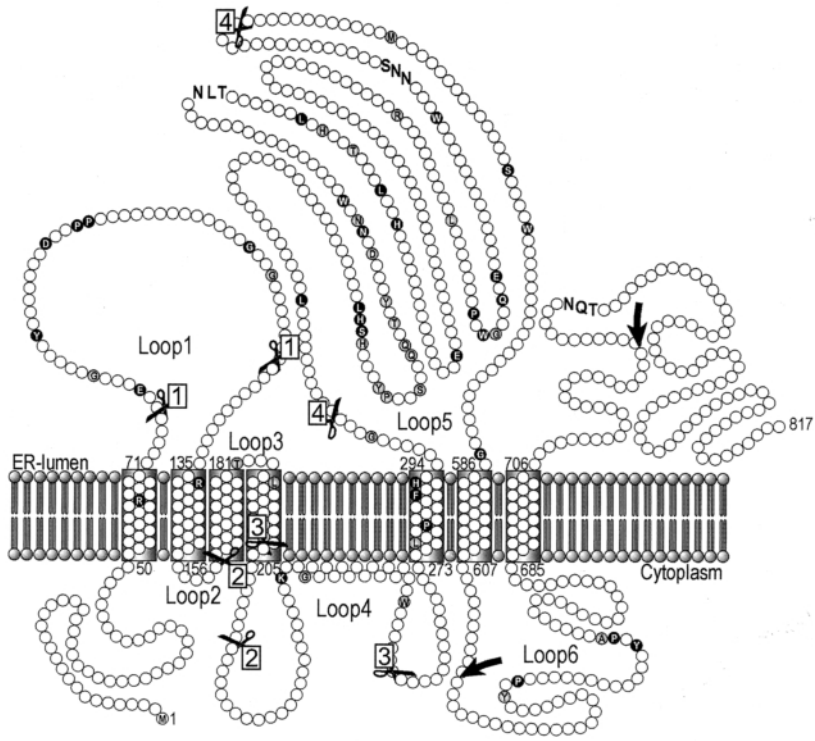


Figure 66 *Schematic presentation of the dolichyl phosphate-mannose:protein* O*-mannosyltransferase (ScPmt1p). ScPmt1p spans the ER membrane seven times. The amino terminus and loops 2, 4 and 6 face the cytoplasm. A putative 'leave-one-out' topology of loop 4 is indicated. The loop 1, loop 3 and loop 5 segments as well as the carboxyl terminus are oriented toward the ER lumen*
(Reproduced with permission from Girrbach *et al.*[194])

S-Glutathionation of proteins adjusts the intracellular redox state and could assist *in vivo* oxidative and nitrosation processes.[196]

11 Other Biological Functions for Proteins

A compost-dwelling earthworm, *Eisenia foetida* (Savigny), has a modest ability to concentrate trace elements and specifically cadmium through its Cd-metallothionein, an essential detoxification strategy. This unexpectedly small (41 residue) protein (Scheme 7) contains 12 cysteine residues that bind four Cd ions, and the protein owes its conformational stability to an adamantane-like three-dimensional arrangement.[197]

Drosomycin is a 44-residue protein from *Drosophila melanogaster* that is the first antifungal protein to be found in an insect species.[198] The solution structure of this protein, a three-stranded β-sheet and an α-helix stabilized by four disulfide bonds, is a motif characteristic of antibacterial insect defensins. This is now

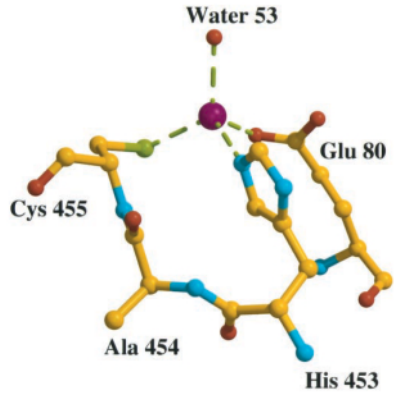


Figure 67 *Close-up view of the zinc coordination at the C-terminal splicing junction of molecule B of VMA29. The purple sphere is a zinc atom*
(Reproduced with permission from Poland *et al.*[195])

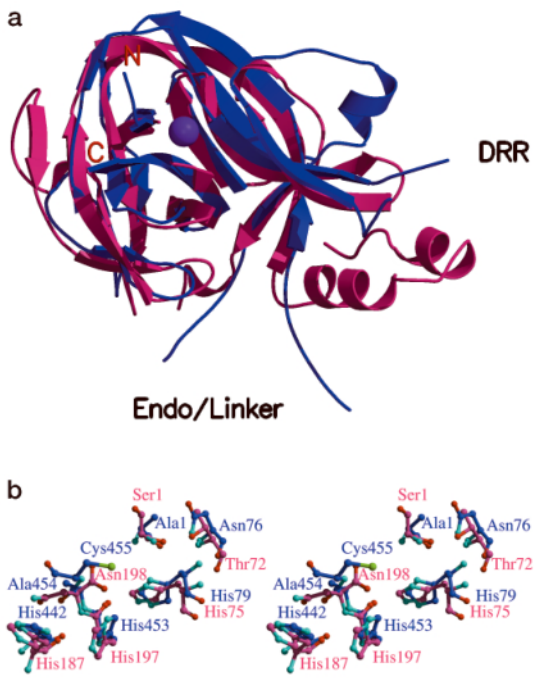


Figure 68 *(a) Superpositioned ribbon diagram of the VMA29 (blue) and GyrA (red) HINT modules. For clarity, the endonuclease domain (Endo/Linker) and DNA recognition region (DRR) of VMA29 were not included in the diagram. The zinc atom from VMA29 is shown as a purple sphere. (b) Stereoview of the superposition of residues involved in protein splicing from VMA29 (blue) and GyrA (red). The superposition was as indicated in (a)*
(Reproduced with permission from Poland *et al.*[195])

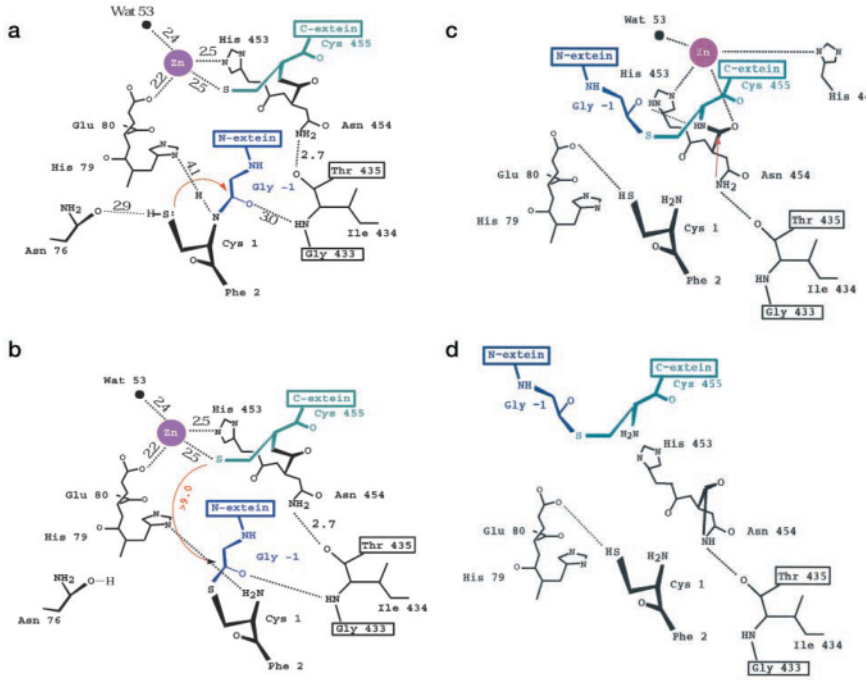


Figure 69 *(a)–(d) Proposed mechanism of protein splicing involving a structural zinc atom. The red arrows indicate points of nucleophilic attack. Blue residues are part of N-extein and aqua residues are part of the C-extein; black residues are part of the intein*
(Reproduced with permission from Poland *et al.*[195])

supplemented by details of the site of its interaction with fungal hyphae (Figure 70).

The cytotoxicity of ribonuclease A is a consequence of its conformational stability (see also ref. 42);[199] it has been suggested that factors that allow avoidance of proteolysis, confer cytotoxicity on a protein.

The two forms of dimeric ribonuclease A have been given new scrutiny; the conformational isomers have been known for many years but were thought to have no structural or functional differences, but they do indeed differ in detail, thus clarifying current misunderstandings.[200]

P-Glycoprotein catalyses the hydrolysis of ATP and couples this process to drug transport. This behaviour is relevant to multidrug resistance since it causes the transport of hydrophilic compounds from cells and prevents the accumulation of cancer drugs and HIV protease inhibitors at particular sites.[201]

12 Viral Proteins

This title refers to the literature covering the protein content of intact virus particles, and the topic is included here to illustrate the application of physical

Scheme 7 *41-Residue peptide from 80-residue prepropeptide (post-translational cleavage peptide of putative gene product coded by the metallothionin cDNA) described in ref. 197*
(After Gruber *et al.*[197])

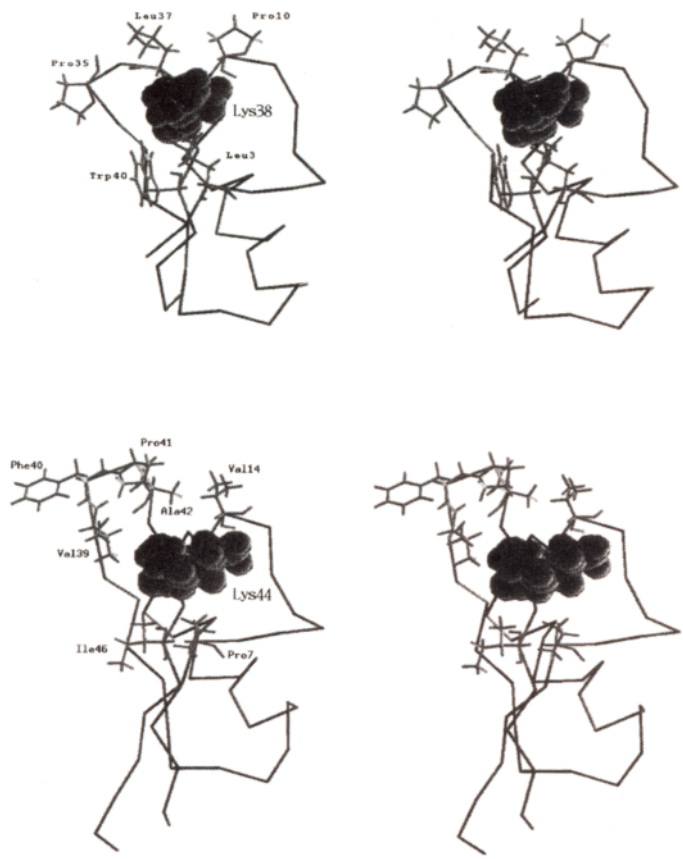


Figure 70 *Hydrophobic clusters containing the basic hydrophobic dyad essential for antifungal activity of drosomycin. The dyad is Lys38–Trp40 in the upper structure and Lys44–Ile46 in Rs-AFP2 in the lower picture*
(Reproduced with permission from Landon *et al.*,[198] *J. Peptide Res.*, Blackwell Science)

methods of structure determination to total biological entities that include protein constituents, even though the protein only makes up a minor part of the whole.

The scale of this task for analytical techniques, and the successes of current structure determination methods, can be appreciated by reference to the estab-

lishment of the structure of the reovirus core, an assembly with relative molecular mass close to 52 million. This entity is essentially a molecular synthesis machine that synthesizes, modifies and exports viral messenger RNA in an operation that takes place within a protein shell. The protein shell appears to be common to all groups of double-stranded RNA viruses.[202]

13 References

1. *Protein Kinase Protocols*, A. D. Reith, ed., Humana Press, Totowa, 2000.
2. *Protein Structure Prediction*: *Methods and Protocols*, D. M. Webster, ed., Humana Press, Totowa, 2000.
3. *Introduction to Protein Architecture*, A. M. Lesk, Oxford University Press, 2000.
4. *Handbook of Metalloproteins*, Vols. 1 and 2, A. Messerschmidt, R. Huber, T. Poulis and K. Wieghardt, eds., Wiley, Chichester, 2001.
5. *Protein Purification*, S. Roe, ed., Oxford University Press, Oxford, 2000 (there are several different books on this topic edited by this author published elsewhere in 2000).
6. *Proteins*, *Peptides and Amino Acids Sourcebook*, J. S. White and D. C. White, eds., Humana Press, Totowa, 2002.
7. *Proteins*: *Biochemistry and Biotechnology*, G. Walsh, Wiley, New York, 2001.
8. *Handbook on Metalloproteins*, I. Bertini, A. Sigel and H. Sigel, eds., Dekker, New York, 2001.
9. *Structure and Mechanism in Protein Science*, Second Ed., A. Fersht, W. H. Freeman, New York, 1999; *Introduction to Protein Structure*, Second Ed., C. Branden and J. Tooze, Garland Publishing, New York, 1999; *Comprehensive Biological Catalysis*, Vols. 1–3, M. L. Sinnott, ed., Academic Press, New York, 1998.
10. *Proteins*, R. H. Angeletti, ed., Academic Press, San Diego, 1998; *Methods for the Investigation of Amino Acid and Protein Metabolism*, A. E. El-Khoury, ed., CRC Press, Boca Raton, Florida, 1999.
11. *Current Advances in Protein Biochemistry*, published by Elsevier (issues appear monthly).
12. *Advances in Protein Chemistry*, Vol. 58, D. Kilnman, ed., Harcourt, New York, 2001.
13. *Introduction to Proteomics*: *Tools for the New Biology*, D. C. Lieber, Humana Press, Totowa, 2001.
14. *Genomics and Proteomics*: *Functional and Computational Aspects*, S. Suhai, ed., Plenum Press, New York, 2000.
15. *From Genome to Proteome*: *Application of Proteomics*, M. J. Dunn, ed., Wiley, New York, 2000.
16. *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins*, A. D. Baxevanis and B. F. Quellette, eds., Wiley, New York, 2001.
17. S. Karlin, A. Bergman and A. J. Gentles, *Nature*, 2001, **411**, 259.
18. G. Folkers and C. D. P. Klein, *Angew. Chem. Int. Ed.*, 2001, **40**, 4175.
19. *Proteome Research*: *Mass Spectrometry* (*Principles and Practice*), P. James, ed., Springer, Heidelberg , 2000.
20. A. Pandey and M. Mann, *Nature*, 2000, **405**, 837.
21. *Mass Spectrometry of Proteins and Peptides*, J. R. Chapman, Humana, Totowa, 2000.

22. *Protein Sequencing and Identification Using Tandem Mass Spectrometry*, M. Kinter and N. E. Sherman, Wiley-Interscience, New York, 2000.
23. B. Bothner, R. Chavez, J. Wei, C. Strupp, Q. Phung, A. Schneemann and G. Siuzdak, *J. Biol. Chem.*, 2000, **275**, 13455.
24. J. Villanueva, F. Canals, V. Villegas, E. Querol and F. X. Aviles, *FEBS Lett.*, 2000, **472**, 27.
25. Y. Yang, K. Janota, K. Tabei, N. Huang, M. M. Siegel, Y.-I. Lin, B. A. Rasmussen and D. M. Shlaes, *J. Biol. Chem.*, 2000, **275**, 26674.
26. M. M. Young, N. Tang, J. C. Hempel, C. M. Oshiro, E. W. Taylor, I. D. Kuntz, B. W. Gibson and G. Dollinger, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 5802.
27. A. Westerholm-Parvinen, I. Vernos and L. Serrano, *FEBS Lett.*, 2000, **486**, 285.
28. *Protein Dynamics Using NMR Relaxation*, K. H. Mayo and V. A. Daragan, Imperial College Press, London, 2001.
29. H. R. Kallitzer, A. Gorler, H. Li, P. V. Dubovskii, W. Hengstenberg, C. Kowolk, H. Yamada and K. Akasaka, *Protein Science*, 2000, **9**, 693.
30. S. Park, M. E. Johnson and L. W.-M. Fung, *FEBS Lett.*, 2000, **485**, 81.
31. J. D. van Beek, L. Beaulieu, H. Schafer, M. Demura, T. A. Sakura and B. H. Meier, *Nature*, 2000, **405**, 1077.
32. G. M. Clore, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 9021.
33. *Protein Folding, Evolution and Design*, R. A. Broglia, E. I. Shakhnovich and G. Tiana, eds., IOS Press, 2001.
34. *From Protein Folding to New Enzymes*, A. Berry and S. Radford, eds., Portland Press, 2001.
35. *Protein Structure, Stability and Folding*, K. P. Murphy, ed., Humana Press, Totowa, 2001.
36. *Mechanisms of Protein Folding*, R. H. Pain, ed., Oxford University Press, 2000.
37. M. M. Barnhart, J. S. Pinkner, G. E. Soto, F. G. Sauer, S. Langermann, G. Waksman, C. Frieden and S. J. Hultgren, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 7709.
38. D. Baker, *Nature*, 2000, **405**, 39.
39. K. A. Diss and H. S. Chan, *Nat. Struct. Biol.*, 1997, **4**, 10.
40. E. Paci and M. Karplus, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6521.
41. H.-J. S. Leiros, N. P. Willassen and A. O. Smalas, *Eur. J. Biochem.*, 2000, **267**, 1039.
42. T. A. Klink, K. J. Woycechowsky, K. M. Taylor and R. T. Raines, *Eur. J. Biochem.*, 2000, **267**, 566.
43. L. K. Low, H.-C. Shin, M. Narayan, W. J. Wedemeyer and H. A. Scheraga, *FEBS Lett.*, 2000, **472**, 67.
44. G. D. Clark-Walker, P. M. Hansbro, F. Gibson and X. J. Chen, *Biochim. Biophys. Acta*, 2000, **1478**, 125.
45. J. Kleinjung, P. Bayley and F. Fraternali, *FEBS Lett.*, 2000, **470**, 257.
46. D. K. Wilkins, C. M. Dobson and M. Gross, *Eur. J. Biochem.*, 2000, **267**, 2609.
47. S. Goda, K. Takano, Y. Yamagata, R. Nagata, H. Akatsu, S. Maki, K. Namba and K. Yutani, *Protein Science*, 2000, **9**, 369.
48. H. Schuler, M. Nyakera, C. E. Schutt, U. Lindberg and R. Karlsson, *FEBS Lett.*, 2000, **476**, 155; see also H. Schuler, C. E. Schutt, U. Lindberg and R. Karlsson, *Eur. J. Biochem.*, 2000, **267**, 476.
49. M. Prabhakaran, M. Dudek, G. Raghunathan and K. Ramnarayan, *J. Peptide Res.*, 2000, **56**, 12.
50. P. L. Yeagle, A. Salloum, A. Chopra, N. Bhawsar, L. Ali, G. Kuzmanovski, J. L. Alderfer and A. D. Albert, *J. Peptide Res.*, 2000, **55**, 455.

51. H.-J. Lee and G.-G. Chang, *Eur. J. Biochem.*, 2000, **267**, 3979.
52. J. Bouckaert, Y. Dewallef, F. Poortmans, L. Wyns and R. Loris, *J. Biol. Chem.*, 2000, **275**, 19778.
53. S. L. De Wall, E. S. Meadows, L. J. Barbour and G. W. Gokel, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6271.
54. M. Eilers, S. C. Shekar, T. Shieh, S. O. Smith and P. J. Fleming, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 5796.
55. C. Lee, S.-H. Park, M.-Y. Lee and M.-H. Yu, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 7727.
56. J. A. Huntington, R. J. Read and R. W. Carrell, *Nature*, 2000, **407**, 923.
57. F. L. García, R. Zahn, R. Riek and K. Wüthrich, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 8334.
58. M. Marino, L. Braun, P. Cossart and P. Ghosh, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 8784.
59. *Amino Acids and Peptides*, G. C. Barrett and D. T. Elmore, Cambridge University Press, 1998, p. 20.
60. T. M. Weaver, *Protein Science*, 2000, **9**, 201.
61. H. Lilie, W. Haehnel, R. Rudolph and U. Baumann, *FEBS Lett.*, 2000, **470**, 173.
62. Y.-C. Liou, A. Tocilj, P. L. Davies and Z. Jia, *Nature*, 2000, **406**, 322.
63. S. P. Graether, M. J. Kulper, S. M. Gagne, V. K. Walker, Z. Jia, B. D. Sykes and P. L. Davies, *Nature*, 2000, **406**, 325.
64. W. R. Taylor, *Nature*, 2000, **406**, 916.
65. S.-J. Cho, M. G. Lee, J. K. Yang, J. Y. Lee, H. K. Song and S. W. Suh, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 8932.
66. *Protein–Protein Interactions*, E. Golemis, ed., Cold Spring Harbour Laboratory Press, USA, 2001.
67. *Protein–Protein Recognition*, C. Kleanthous, ed., Oxford University Press, 2000.
68. *RNA-Binding Proteins*, K. Sandberg and S. E. Mulroney, eds., Kluwer, Dordrecht, 2001.
69. P. Uetz, L. Giot, M. Cagney, T. A. Mansfield, R. S. Judson, J. R. Knight, D. Lockshon, V. Narayan, M. Srinivasan, P. Pochart, A. Qureshi-Emili, Y. Li, B. Godwin, D. Conover, T. Kalbfleisch, G. Vijayadamodar, M. Yang, M. Johnston, S. Fields and J. M. Rothberg, *Nature*, 2000, **403**, 623.
70. C. T. Rollins, V. M. Rivera, D. N. Woolfson, T. Keenan, M. Hatada, S. E. Adams, L. J. Andrade, D. Yaeger, M. R. van Schravendijk, D. A. Holt, M. Gilman and T. Clackson, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 7096.
71. K. Wang and A. Spector, *Eur. J. Biochem.*, 2000, **267**, 4705.
72. T. M. Treweek, R. A. Lindner, M. Mariani and J. A. Carver, *Biochim. Biophys. Acta*, 2000, **1481**, 175.
73. K. Berr, D. Wassenberg, H. Lilie, J. Behlke and R. Jaenicke, *Eur. J. Biochem.*, 2000, **267**, 5413.
74. T. V. O'Halloran and V. C. Culotta, *J. Biol. Chem.*, 2000, **275**, 25057.
75. J. Tyedmers, M. Kruse, M. Lerner, J. Demand, J. Hohfeld, J. Solsbacher, J. Volkmer and R. Zimmermann, *Eur. J. Biochem.*, 2000, **267**, 3575.
76. J. R. Hoskins, S. K. Singh, M. R. Maurizi and S. Wickner, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 8892.
77. B. Lenarcic, G. Krishnan, R. Borukhovich, B. Ruck, V. Turk and E. Moczydlowski, *J. Biol. Chem.*, 2000, **275**, 15572.
78. A. S. Andersen, P. H. Hansen, L. Schaffer and C. Kristensen, *J. Biol. Chem.*, 2000, **275**, 16948.

79. E. M. Clerico, S. G. Peisajovich, M. Ceolin, P. D. Ghiringhelli and M. R. Ermacora, *Biochim. Biophys Acta*, 2000, **1476**, 203.
80. C.-S. Tung, M. E. Wall, S. C. Gallagher and J. Trewhella, *Protein Science*, 2000, **9**, 1312.
81. L. Wagner, O. Oliyarnyk, W. Gartner, P. Nowotny, M. Groeger, K. Kaserer, W. Waldhäusl and M. S. Pasternack, *J. Biol. Chem.*, 2000, **275**, 24700.
82. M. Mariotti, L. De Bemedictis, E. Avon and J. A. M. Maier, *J. Biol. Chem.*, 2000, **275**, 24047.
83. B. Mehul, D. Bernard, L. Simonetti, M. A. Bernard and R. Schmidt, *J. Biol. Chem.*, 2000, **275**, 12841.
84. X.-S. Gong, J. Q. Wen and J. C. Gray, *Eur. J. Biochem.*, 2000, **267**, 1732.
85. P. Sondermann, R. Huber, V. Oosthuizen and U. Jacob, *Nature*, 2000, **406**, 267.
86. S. C. Garman, B. A. Wurzburg, S. S. Tarchevskaya, J.-P. Kinet and T. S. Jardetzky, *Nature*, 2000, **404**, 259.
87. L. Pellegrini, D. F. Burke, F. von Delft, B. Mulloy and T. L. Blundell, *Nature*, 2000, **407**, 1029.
88. D. M. Hoover, C. S. Mizone, T. M. Handel and J. Lubkowski, *J. Biol. Chem.*, 2000, **275**, 23187.
89. T. K. Dam, B. S. Cavada, T. B. Grangiero, C. F. Santos, V. M. Ceccatto, F. A. M. de Sousa, S. Oscarson and C. F. Brewer, *J. Biol. Chem.*, 2000, **275**, 16119.
90. A. Imberty, C. Gautier, J. Lescar, S. Perez, L. Wyns and R. Loris, *J. Biol. Chem.*, 2000, **275**, 17541.
91. C. Liu, E. Gelius, G. Liu, H. Steiner and R. Dziarski, *J. Biol. Chem.*, 2000, **275**, 24490.
92. R. Chiaraluce, V. Consalvi, S. Cavallo, A. Ilari, S. Stefanini and E. Chiancone, *Eur. J. Biochem.*, 2000, **267**, 5733.
93. L. Jin, S. L. Briggs, S. Chandrasekhar, N. Y. Chirgadze, D. K. Clawson, R. W. Schevitz, D. L. Smiley, A. H. Tashjian and F. Zhang, *J. Biol. Chem.*, 2000, **275**, 27238.
94. *Proteolytic Enzymes*, R. Beynon and J. S. Bond, eds., Oxford University Press, 2001.
95. *Tyrosine Phosphoprotein Phosphatases*, Second Ed., B. J. Goldstein, Oxford University Press, 1998.
96. C. H. Williams, *Eur. J. Biochem.*, 2000, **267**, 6101; E. S. J. Arner and A. Holmgren, *Eur. J. Biochem.*, 2000, **267**, 6102; C. H. Williams, L. D. Arscott, S. Muller, B. W. Lennon, M. L. Ludwig, P.-F. Wang, D. M. Veine, K. Becker and R. H. Schirmer, *Eur. J. Biochem.*, 2000, **267**, 6110; K. Becker, S. Gromer, R. H. Schirmer and S. Muller, *Eur. J. Biochem.*, 2000, **267**, 6118; L. C. Poole, C. M. Reynolds, Z. A. Wood, P. A. Karplus, H. R. Ellis and M. L. Calzi, *Eur. J. Biochem.*, 2000, **267**, 6126.
97. G. Nicastro, C. de Chiara, E. Pedone, M. Tato, M. Rossi and S. Bartolucci, *Eur. J. Biochem.*, 2000, **267**, 403.
98. L. Zhong and A. Holmgren, *J. Biol. Chem.*, 2000, **275**, 18121.
99. P. G. Board, M. Coggan, G. Chelvanayagam, S. Easteal, L. S. Jermiin, G. K. Schulte, D. E. Danley, L. R. Hoth, M. C. Griffor, A. V. Kamath, M. H. Rosner, B. A. Chrunyk, D. E. Perregaux, C. A. Gabel, K. F. Geoghegan and J. Pandit, *J. Biol. Chem.*, 2000, **275**, 24798.
100. W. T. Lowther, N. Brot, H. Weissbach, J. F. Honek and B. W. Matthews, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6463.
101. J. R. Gillespie and V. N. Uversky, *Biochim. Biophys. Acta*, 2000, **1480**, 41.
102. I. Przylas, Y. Terada, K. Fujii, T. Takaha, W. Saenger and N. Strater, *Eur. J. Biochem.*, 2000, **267**, 6903.

103. M. Scharpf, H. Sticht, K. Schweimer, M. Boehm, S. Hoffmann and P. Rosch, *Eur. J. Biochem.*, 2000, **267**, 2397.
104. M. Sugishima, Y. Omata, Y. Kakuka, H. Sakamoto, M. Noguchi and K. Fukuyama, *FEBS Lett.*, 2000, **471**, 61.
105. C. Savino, L. Federici, R. Ippoliti, E. Lendaro and D. Tsernoglou, *FEBS Lett.*, 2000, **470**, 239.
106. A. Kuglstatter, J. Miksovska, P. Sebban and G. Fritzsch, *FEBS Lett.*, 2000, **472**, 114.
107. R. Dua, S. Edwards, D. L. Levy and J. L. Campbell, *J. Biol. Chem.*, 2000, **275**, 28816.
108. G. Lu, D. Dobritzsch, S. Baumann, G. Schneider and S. Konig, *Eur. J. Biochem.*, 2000, **267**, 861.
109. K.-C. Chou, A. G. Tomasselli and R. L. Heinrikson, *FEBS Lett.*, 2000, **470**, 249.
110. M. Yun, C.-G. Park, J.-Y. Kim, C. O. Rock, S. Jackowski and H.-W. Park, *J. Biol. Chem.*, 2000, **275**, 28093.
111. G. Valentini, L. Chiarelli, R. Fortin, M. L. Speranza, A. Galizzi and A. Mattevi, *J. Biol. Chem.*, 2000, **275**, 18145.
112. W. Yan, F. Wu, J. Morser and Q. Wu, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 8525.
113. M. Harel, G. Kryger, T. L. Rosenberry, W. D. Mallender, T. Lewis, R. J. Fletcher, J. M. Guss, I. Silman and J. L. Sussman, *Protein Science*, 2000, **9**, 1063.
114. J. Benach, S. Atrian, J. Fibla, R. Gonzalez-Duarte and R. Ladenstein, *Eur. J. Biochem.*, 2000, **267**, 3613.
115. D. H. Juers, R. H. Jacobson, D. Wigley, X.-J. Zhang, R. E. Huber, D. E. Tronrud and B. W. Matthews, *Protein Science*, 2000, **9**, 1685.
116. W. J. Cook, L. J. DeLucas and S. Chattopadhy, *Protein Science*, 2000, **9**, 704.
117. N. E. Labrou, D. J. Rigden and Y. D. Clonis, *Eur. J. Biochem.*, 2000, **267**, 6657.
118. B. K. Mauralidhara and M. Hirose, *Protein Science*, 2000, **9**, 1567.
119. S. Kreusch, M. Fehn, G. Maubach, N. Kissler, W. Rommerskirch, K. Schilling, E. Weber, I. Wenz and B. Wiederanders, *Eur. J. Biochem.*, 2000, **267**, 2965.
120. S. Ghosh, R. Brown, J. C. R. Jones, S. M. Ellerbroek and M. S. Stack, *J. Biol. Chem.*, 2000, **275**, 23869.
121. E. S. Harris, T. M. McIntyre, S. M. Prescott and G. A. Zimmermann, *J. Biol. Chem.*, 2000, **275**, 23409.
122. Y. Luo, E. A. Frey, R. A. Pfuetzner, A. L. Creagh, D. G. Knoechel, C. A. Haynes, B. B. Finlay and N. C. J. Strynadka, *Nature*, 2000, **405**, 1073.
123. P. C. Bourne, M. N. Isupov and J. A. Littlechild, *Structure*, 2000, **8**, 143.
124. M. Kvaratskhelia, B. N. Wardleworth, D. G. Norman and M. F. White, *J. Biol. Chem.*, 2000, **275**, 25540.
125. Y. Liu and D. V. Santi, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 8263.
126. C. J. Hawkins, S. J. Yoo, E. P. Peterson, S. L. Wang, S. Y. Vernooy and B. A. Hay, *J. Biol. Chem.*, 2000, **275**, 27084.
127. F. Francois, M. J. Godinho and M. L. Grimes, *FEBS Lett.*, 2000, **486**, 281.
128. P. J. Ellis, C. K. S. Carlow, D. Ma and P. Kuhn, *Biochemistry*, 2000, **39**, 592.
129. X.-F. Tang, S. Ezaki, H. Atomi and T. Imanaka, *Eur. J. Biochem.*, 2000, **267**, 6369.
130. S. Y. K. Seah, G. Labbe, S. Nerdinger, M. R. Johnson, V. Suleckus and L. D. Eltis, *J. Biol. Chem.*, 2000, **275**, 15701.
131. W. J. Peumans, A. Barre, V. Derycke, P. Rouge, W. Zhang, G. D. May, J. A. Delcour, F. Van Leuven and E. J. M. Van Damme, *Eur. J. Biochem.*, 2000, **267**, 1188.
132. W. T. Lowther and B. W. Matthews, *Biochim. Biophys. Acta*, 2000, **1477**, 157.
133. K.-H. Park, T.-J. Kim, T.-K. Cheong, J.-W. Kim, B.-H. Oh and B. Svensson,

Biochim. Biophys. Acta, 2000, **1478**, 165.
134. J. M. Bragger, R. V. Dunn and R. M. Daniel, *Biochim. Biophys. Acta*, 2000, **1480**, 278.
135. X.-P. Huang, N. Kagami, H. Inoue, M. Kojima, T. Kimura, O. Makabe, K. Suzuki and K. Takahashi, *J. Biol. Chem*., 2000, **275**, 26607.
136. K. S. Kim, K. S. Oh and J. Y. Lee, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6373.
137. H. J. Chauhan, G. J. Domingo, H.-I. Jung and R. N. Perham, *Eur. J. Biochem*., 2000, **267**, 7158.
138. S. P. Webster, D. Alexeev, D. J. Campopiano, R. M. Watt, M. Alexeeva, L. Sawyer and R. L. Baxter, *Biochemistry*, 2000, **39**, 516.
139. J. W. Rausch, B. K. Sathyanarayana, M. K. Bona and S. F. Le Grice, *J. Biol. Chem*., 2000, **275**, 16015.
140. A. Daiber, S. Herold, C. Schoneich, D. Namgaladze, J. A. Peterson and V. Ullrich, *Eur. J. Biochem*., 2000, **267**, 6729.
141. T. Yamada, C. Morisseau, J. E. Maxwell, M. A. Argiriadi, D. W. Christianson and B. D. Hammock, *J. Biol. Chem*., 2000, **275**, 23082.
142. M. Elde, N. P. Willassen and S. Johnson, *Eur. J. Biochem*., 2000, **267**, 7257.
143. H. Schuler, M. Nyakeru, C. E. Schutt, U. Lindberg and R. Karlsson, *Eur. J. Biochem*., 2000, **267**, 4054.
144. L. W. Ruddock, R. B. Freedman and P. Klappa, *Protein Science*, 2000, **9**, 758.
145. M. W. Bader, T. Xie, C.-A. Yu and J. C. A. Bardwell, *J. Biol. Chem*., 2000, **275**, 26082.
146. L. Chung, K. Shimokawa, D. Dinakarpandian, F. Grams, G. B. Fields and H. Nagase, *J. Biol. Chem*., 2000, **275**, 29610.
147. K. S. Hewitson, J. E. Baldwin, N. M. Shaw and P. L. Roach, *FEBS Lett*., 2000, **466**, 372; see also L. McIver, R. L. Baxter and D. J. Campopiano, *J. Biol. Chem*., 2000, **275**, 13888.
148. J. R. Kiefer, J. L. Pawlitz, K. T. Moreland, R. A. Stegeman, W. F. Hood, J. K. Gierse, A. M. Stevens, D. C. Goodwin, S. W. Rowlinson, L. J. Marnett, W. C. Stallings and R. G. Kurumbail, *Nature*, 2000, **405**, 97.
149. M. H. F. Sorgine, C. Logullo, R. B. Zingali, G. O. Paiva-Silva, L. Juliano and P. L. Oliveira, *J. Biol. Chem*., 2000, **275**, 28659.
150. R. J. Darling, R. W. Ruddon, F. Perini and E. Bedows, *J. Biol. Chem*., 2000, **275**, 15413.
151. A. A. Vostrov and W. W. Quitschke, *J. Biol. Chem*., 2000, **275**, 22978.
152. M. C. Pearce, H. Rubin and S. P. Bottomley, *J. Biol. Chem*., 2000, **275**, 28513.
153. Y. Ikari, K. Fujikawa, K. O. Yee and S. M. Schwartz, *J. Biol. Chem*., 2000, **275**, 12279.
154. S. P. Bottomley and D. J. Tens, *Biochim. Biophys. Acta*, 2000, **1481**, 11.
155. J. A. Huntington, A. McCoy, K. J. Belzar, X. Y. Pei, P. G. W. Gettins and R. W. Carrell, *J. Biol. Chem*., 2000, **275**, 15377.
156. H. Im and M.-H. Yu, *Protein Science*, 2000, **9**, 934.
157. P. R. Elliott, X.-Y. Pei, T. R. Dafforn and D. A. Lomas, *Protein Science*, 2000, **9**, 1274.
158. H. Im, H.-Y. Ahu, and M.-H. Yu, *Protein Science*, 2000, **9**, 1497.
159. O. L. Franco, D. J. Rigden, F. R. Melo, C. Bloch, C. P. Silva and M. F. Grossi de Sa, *Eur. J. Biochem*., 2000, **267**, 2166.
160. K. Hiraga, T. Suzuki and K. Oda, *J. Biol. Chem*., 2000, **275**, 25173.
161. J. Brzin, B. Rogelj, T. Popovic, B. Strukelj and A. Ritonja, *J. Biol. Chem*., 2000, **275**, 20104.

162. J. Guay, J.-P. Falgueyret, A. Ducret, M. D. Percival, and J. A. Mancini, *Eur. J. Biochem.*, 2000, **267**, 6311.
163. L. Camardella, U. Carratore, M. A. Ciardello, L. Servillo, C. Balastrieri and A. Giovane, *Eur. J. Biochem.*, 2000, **267**, 4561.
164. S. G. Kang, H. U. Park, H. S. Lee, H. T. Kim and K. J. Lee, *J. Biol. Chem.*, 2000, **275**, 16851.
165. P. R. E. Mittl, L. Lutley, P. Hunziker and M. G. Grutter, *J. Biol. Chem.*, 2000, **275**, 17693.
166. W. Huang, Z. Zhang and T. Palzkill, *J. Biol. Chem.*, 2000, **275**, 14964.
167. C. Therrien, L. P. Kotra, F. Sanschagrin, S. Mobashery and R. C. Levesque, *FEBS Lett.*, 2000, **470**, 285.
168. E. J. M. Van Damme, Q. Hao, D. Charels, A. Barre, P. Rouge, F. Van Leuven and W. J. Peumans, *Eur. J. Biochem.*, 2000, **267**, 2746.
169. A. M. Milstone, L. M. Harrison, R. D. Bungiro, P. Kuzmic and M. Cappello, *J. Biol. Chem.*, 2000, **275**, 29391.
170. R. Conde, F. Z. Zamudio, M. H. Rodriguez and L. D. Possani, *FEBS Lett.*, 2000, **471**, 165.
171. R. C. Padre and J. T. Stull, *FEBS Lett.*, 2000, **472**, 148.
172. A. Radominska-Pandya, G. Chen, P. J. Czernik, J. M. Little, V. M. Samokyszyn, C. A. Carter and G. Nowak, *J. Biol. Chem.*, 2000, **275**, 22324.
173. A. G. Tomasselli and R. L. Heinrikson, *Biochim. Biophys. Acta*, 2000, **1477**, 189; see also S. Todd, C.-G. Anderson, D. J. Jolly and C. S. Craik, *Biochim. Biophys. Acta*, 2000, **1477**, 168.
174. D. Lee, S. A. Long, J. L. Adams, G. Chan, K. S. Vaidya, T. A. Francis, K. Kikly, J. D. Winkler, C.-M. Sung, C. Debouck, S. Richardson, M. A. Levy, W. E. DeWolf, P. M. Keller, T. Tomaszek, M. S. Head, M. D. Ryan, R. C. Haltiwanger, P.-H. Liang, C. A. Janson, P. J. McDevitt, K. Johanson, N. O. Concha, W. Chan, S. S. Abdel-Meguid, A. M. Badger, M. W. Lark, D. P. Nadeau, L. J. Suva, M. Gowen and M. E. Nuttall, *J. Biol. Chem.*, 2000, **275**, 16007.
175. H. Mehmet, *Nature*, 2000, **403**, 29.
176. T. Nakagawa, H. Zhu, N. Morishima, E. Li, J. Xu, B. A. Yankner and J. Yuan, *Nature*, 2000, **403**, 98.
177. *Ann. N. Y. Acad. Sci.*, 2000, Vol. 924.
178. M. S. Dennis, C. Eigenbrot, N. J. Skelton, M. H. Ultsch, L. Santell, M. A. Dwyer, M. P. O'Connell and R. A. Lazarus, *Nature*, 2000, **404**, 465.
179. P. Fuentes-Prior, Y. Iwanaga, R. Huber, R. Pagila, G. Rumennik, M. Seto, J. Morser, D. R. Light and W. Bode, *Nature*, 2000, **404**, 518.
180. J. Torres and I. T. Arkin, *Eur. J. Biochem.*, 2000, **267**, 3422.
181. M. R. Stofega, L. S. Argetsinger, H. Wang, A. Ullrich and C. Carter-Su, *J. Biol. Chem.*, 2000, **275**, 28222.
182. G. H. Peters, L. F. Iversen, S. Branner, H. S. Andersen, S. B. Mortensen, O. H. Olsen, K. B. Moller and N. P. H. Moller, *J. Biol. Chem.*, 2000, **275**, 18201.
183. P. V. Dubovskii, H. Li, S. Takahashi, A. S. Arseniev and K. Akasaka, *Protein Science*, 2000, **9**, 786.
184. C. A. Saura, T. Tomita, S. Soriano, M. Takahashi, J.-Y. Leem, T. Honda, E. H. Koo, T. Iwatsubo and G. Thinakaran, *J. Biol. Chem.*, 2000, **275**, 17136.
185. E. Pack-Chung, M. B. Meyers, W. P. Pettingell, R. D. Moir, A. M. Brownewell, I. Cheng, R. E. Tanzi and T.-W. Kim, *J. Biol. Chem.*, 2000, **275**, 14440.
186. Y.-M. Li, M.-T. Lai, M. Xu, Q. Huang, J. DiMuzio-Mower, M. K. Sardana, X.-P. Shi, K.-C. Yin, J. A. Shafer and S. J. Gardell, *Proc. Natl. Acad. Sci. USA*, 2000, **97**,

6138.
187. L. Kulic, J. Walter, G. Multhaup, D. B. Teplow, R. Baumeister, H. Romig, A. Capell, H. Steiner and C. Haass, *Proc. Natl. Acad. Sci. USA*, 2000, 97, 5913.
188. D. J. Selkoe and M. S. Wolfe, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 5690.
189. W. Xia, W. J. Ray, B. L. Ostaszewski, T. Rahmati, W. T. Kimberly, M. S. Wolfe, J. Zhang, A. M. Goate and D. J. Selkoe, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 9299.
190. K. M. S. Misura, R. H. Scheller and W. I. Weis, *Nature*, 2000, **404**, 355.
191. A. Royant, K. Edman, T. Ursby, E. Pebay-Peyroula, E. M. Landau and R. Neutze, *Nature*, 2000, **406**, 645; H. J. Sass, G. Buldt, R. Gessenich, D. Hehn, D. Neff, R. Schlesinger, J. Berendzen and P. Ormos, *Nature*, 2000, **406**, 649; S. Subramaniam and R. Henderson, *Nature*, 2000, **406**, 653; see also W. Kuhlbrandt, *Nature*, 2000, **406**, 569.
192. C. Loske, A. Gerdemann, W. Schepl, M. Wycislo, R. Schinzel, D. Palm, P. Riederer and G. Munch, *Eur. J. Biochem.*, 2000, **267**, 4171.
193. M. Schlumpberger, H. Wille, M. A. Baldwin, D. A. Butler, I. Herskowitz and S. B. Prusiner, *Protein Science*, 2000, **9**, 440.
194. V. Girrbach, T. Zeller, M. Priesmeier and S. Strahl-Bolsinger, *J. Biol. Chem.*, 2000, **275**, 19288.
195. B. W. Poland, M.-Q. Xu and F. A. Quiocho, *J. Biol. Chem.*, 2000, **275**, 16408.
196. P. Klatt and S. Lamas, *Eur. J. Biochem.*, 2000, **267**, 4928.
197. C. Gruber, S. Sturzenbaum, P. Gehrig, R. Sack, P. Hunziker, B. Berger and R. Dallinger, *Eur. J. Biochem.*, 2000, **267**, 573.
198. C. Landon, A. Pajon, F. Vovelle and P. Sodano, *J. Peptide Res.*, 2000, **56**, 231.
199. T. A. Klink and R. T. Raines, *J. Biol. Chem.*, 2000, **275**, 17463.
200. S. Sorrentino, R. Barone, E. Bucci, G. Gotte, N. Russo, M. Libonati and G. D'Alessio, *FEBS Lett.*, 2000, **466**, 35.
201. I. L. Urbatsch, K. Gimi, S. Wilke-Mounts and A. E. Senior, *J. Biol. Chem.*, 2000, **275**, 25031.
202. K. M. Reinisch, M. L. Nibert and S. C. Harrison, *Nature*, 2000, **404**, 960.

In an ever-increasing domain of activity, **Amino Acids, Peptides and Proteins** provides an annual compilation of the world's research effort into this important area of biological chemistry. Comprising a comprehensive review of significant developments at this biology/chemistry interface, each volume opens with an overview of amino acids and their applications. Work on peptides is reviewed over several chapters, ranging from current trends in their synthesis and conformational and structural analysis, to peptidomimetics and the discovery of peptide-related molecules in nature. The application of advanced techniques in structural elucidation is incorporated into all chapters, whilst periodic chapters on metal complexes of amino acids, peptides and beta-lactams extend the scope of coverage.

Efficient searching of specialist topics is facilitated by the sub-division of chapters into discrete subject areas, allowing annual trends to be monitored. All researchers in the pharmaceutical and allied industries, and at the biology/chemistry interface in academia will find this an indispensable reference source.

ISBN 0-85404-237-7

SPECIALIST PERIODICAL REPORTS

Specialist Periodical Reports provide systematic and detailed review coverage in major areas of chemical research. Compiled by teams of leading authorities in the relevant subject areas, the series creates a unique service for the active research chemist, with regular, in-depth accounts of progress in particular fields of chemistry. Subject coverage within different volumes of a given title is similar and publication is on an annual or biennial basis.

Current subject areas covered are *Amino Acids, Peptides and Proteins*, *Carbohydrate Chemistry*, *Catalysis*, *Chemical Modelling: Applications and Theory*, *Electron Paramagnetic Resonance*, *Nuclear Magnetic Resonance*, *Organometallic Chemistry*, *Organophosphorus Chemistry*, *Photochemistry* and *Spectroscopic Properties of Inorganic and Organometallic Compounds*. From time to time, the series has altered according to the fluctuating degrees of activity in the various fields, but these volumes remain a superb reference point for researchers.