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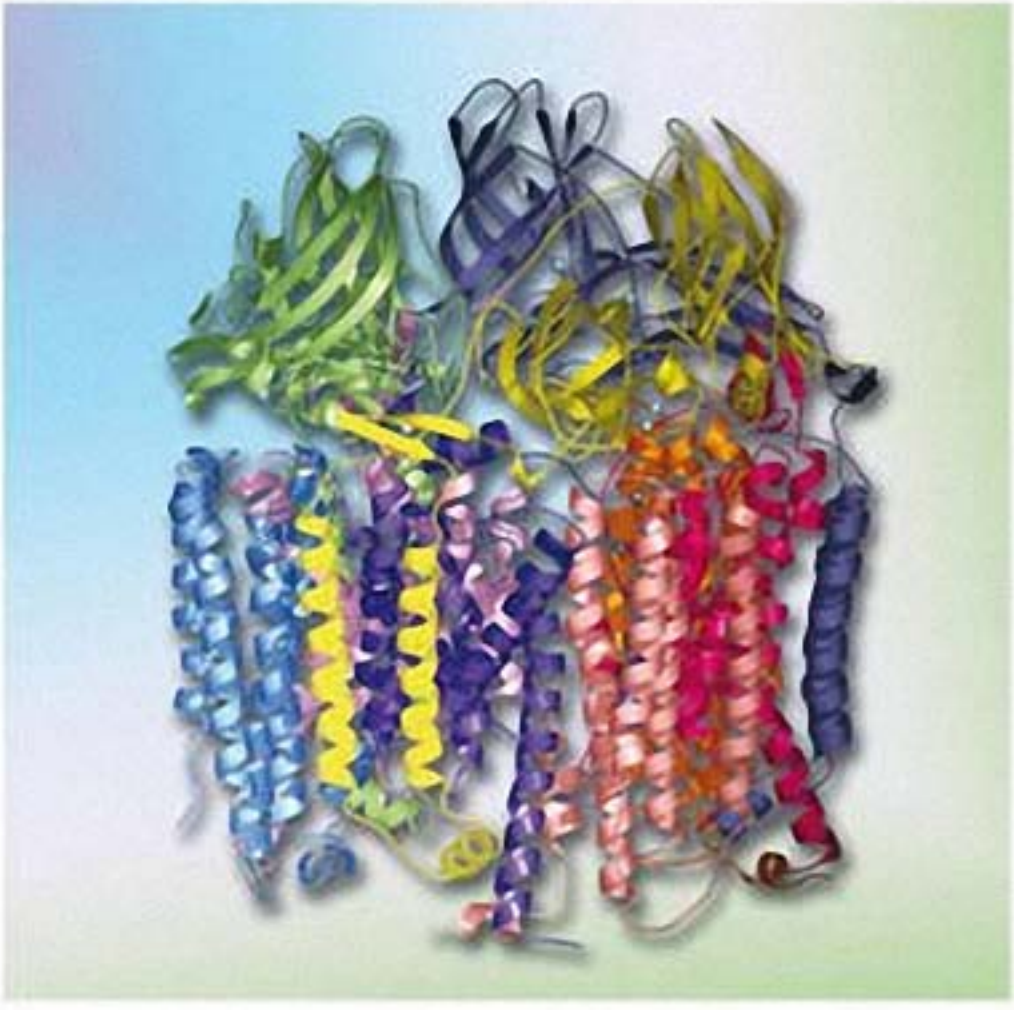
Editor John S Davies

Amino Acids, Peptides and Proteins

Volume 36



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Volume 36

A Specialist Periodical Report

Amino Acids, Peptides and Proteins

Volume 36

A Review of the Literature Published during 2003–2004.

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Preface

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Dr Geoffrey Young, as editor of the first volume in this series in 1968, commented that 'one could hardly ask for a more exciting time at which to review the field'. As a Chapter author, since those early days, I have seen the exciting times continue and the many volumes in this series have been party to great developments in the field. Solid phase synthesis, in its infancy in 1968, has revolutionised the making and manufacturing of peptides and no doubt the number and complexity of the peptides being made these days can only have been dreamt of 40 years ago. The availability of cloned proteins has made available molecular receptors, that now can routinely be used in molecular recognition studies, so that the development of efficient inhibitors has a much more rational basis. The principles of solid phase peptide synthesis have spawned not only a new approach to enhancing the pool of peptides available, but also the discipline of combinatorial chemistry. Numerous novel amino acids have been identified, and research in molecular recognition has increased the demand for novel non-proteinogenic amino acids. Thus developments in asymmetric synthesis have found excellent opportunities in the amino acid context, whose syntheses now depend less on the traditional resolution of synthesised racemates.

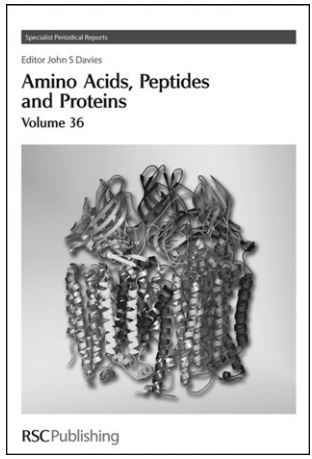
These Specialist Reports have co-existed with the establishment of major international peptide societies, the American, European and Japanese Peptide Societies, and their associated Journals. These have been instrumental in promoting the developments in amino acids, peptides and proteins across a very broad spectrum of activity that transcends the frontiers between chemistry and biology.

This vast expansion of the field has inevitably placed a great deal more pressure on the reporting authors. Yes, computational scanning of the literature has aided the harvesting of papers, but has done little to assist the important phase of placing in context the true significance of the developments in the field. This is a burden that has little recognition, so as I pass on the mantle of Senior Reporter to others, I empathise with and salute the hard work of colleagues who have given long hours of endeavour to produce chapters that are available for you to read within the covers of these SPRs.

This particular Volume has aimed to bring the review coverage up to the end of 2004, and therefore concentrates on the publications of 2003–4. This has brought more pressures of space on the authors, which has meant more selectivity in the selection of papers for review. The recent years have coincided with the ebbing of peptide research in the UK, with fewer research groups from which review authors could be drawn. This Volume has been made possible by the significant input of Hungarian colleagues, Botond Penke, Gábor Tóth Györgyi Váradi, Marta Zarandi, Etelka Farkas and Imre Sóvágó augmenting the continuing efforts of Donald Elmore and John Davies over many years. We all hope that this SPR offers a true reflection of the vast area of activity worldwide. We would have preferred a better time frame to publication, but critical delays have been brought about by pressure of work.

We have aimed to promote good standards of nomenclature, so we have been very grateful for Dr John Jones's unceasing effort to preserve standards and invite the best from others. We are grateful for his willingness to allow his contributions to be compiled within the many Volumes of this series. We also appreciate the assistance of RSC Publishing staff, and we can only hope that the series in its own way contributes to the exciting developments in this field, and prevented many a 'rediscovery of the wheel', which can happen, if the literature is not adequately and thoroughly surveyed.

John S. Davies
Swansea, 2007



Cover

The crystal structure of particulate methane monooxygenase (pMMO) reveals many unexpected features including, a trimeric oligomerization state and three distinct metal centers. Image reproduced by permission of Amy Rosenzweig from *Dalton Transactions*, 2005.

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

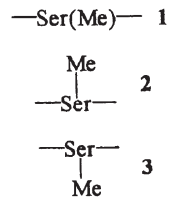
Aad	α-Aminoadipic acid
βAad	β-Aminoadipic acid
Abu	α-Aminobutyric acid
Aib	α-Aminoisobutyric acid; α-methylalanine
βAla	β-Alanine; 3-aminopropionic acid (avoid Bal)
Asu	α-Aminosuberic acid
Aze	Azetidine-2-carboxylic acid
Cha	β-cyclohexylalanine
Cit	Citrulline; 2-amino-5-ureidovaleric acid
Dha	Dehydroalanine (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 symbolism 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 groupis 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

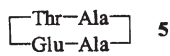
group by writing H on the right is also wrong because that implies a terminal aldehyde.

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**.

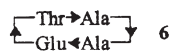


Note that the oxygen atom is not shown: it is contained in the three-letter symbol – showing it, as in Ser(OMe), would imply that a peroxy group was present. Bonds up or down should be used only for indicating side-chain substitution. Confusions may creep in if the three-letter symbols are used thoughtlessly 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**.

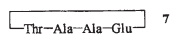
But this is wrong because the left hand vertical bond implies an ester link between the two side chains, and strictly speaking if the right hand vertical bond means anything it means that the two Ala α-carbons are linked by a CH_2CH_2 bridge. This objection could be circumvented by writing the structure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

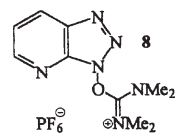
Tfa	Trifluoroacetyl
Tos	4-Toluenesulphonyl (also Ts)
Troc	2,2,2-Trichloroethoxycarbonyl
Trt	Trityl, triphenylmethyl
Xan	9-Xanthydryl
Z	Benzyloxycarbonyl (also Cbz). Z(2Cl) = 2-chlorobenzyl-oxycarbonyl and so on

Amino Acid Derivatives

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

Reagents and Solvents

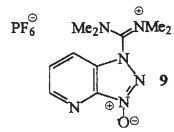
BOP	1-Benzotriazolyloxy-tris-dimethylamino-phosphonium hexafluorophosphate
CDI	Carbonyldiimidazole
DBU	Diazabicyclo[5.4.0]-undec-7-ene
DCCI	Dicyclohexylcarbodiimide (also DCC)
DCHU	Dicyclohexylurea (also DCU)
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate (DMAD = the dimethyl analogue)
DIPCI	Diisopropylcarbodiimide (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′-dimethylamino-propyl)-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

Amino acids

Marta Zarandi

DOI: 10.1039/b700152p

1. Introduction

The occurrence, chemistry, and analysis of amino acids contained in the literature of 2003 and partly 2004 are reviewed in this Chapter which is arranged in sections similar to previous Volumes in this Specialist Periodical report. Scientific Papers published during 2003 (and 2004) have been sourced mainly from the Web of Science databases[1,2] on the internet and from scanning a selection of major journals.

2. Naturally occuring amino acids

2.1 Occurrence of amino acids in nature

Amino acids have been produced with the aid of microorganisms for nearly 50 years. The economic importance of these cellular building blocks is significant. When compared to chemical methods, fermentative production has the advantage of yielding the optically active and biologically required L-form of amino acids from cheap carbon and nitrogen sources. In the review of Tryfona and Bustard,[3] a brief historic background of Coryneform bacteria, which are central to the industrial production of amino acids and the various strategies used for strain improvement are discussed.

Acidic methanolic, whole body extracts of larval *Tenebrio molitor* (Insecta, Coleoptera) and other juvenile insects are highly toxic to adults of the same species and other species: injection causes instant paralysis to death. Referring to their dramatic effect in mature insects, the responsible compounds have been designated as ''paralysins''. Two paralysins have already been identified in the flesh fly, *Neobellieria bullata*, *i.e.*, β-alanyl-tyrosine (BAY) and 3-hydroxykynurenine (3HK). The isolation of two additional paralysins from larval *T. molitor* was reported: (i) the essential amino acid, Trp and (ii) the saturated β-carboline, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA).[4]

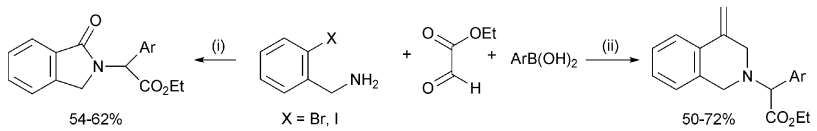
2.2 New amino acids and derivatives

In recent years there has been an increasing interest in new methods that access novel non-proteinogenic α-amino acid derivatives. Although many routes to amino acids have been developed, the Petasis reaction provides a concise and convergent approach that allows structure variability and facile incorporation of functional groups. A novel one pot Petasis reaction/palladium catalysed process is described involving 2-iodo/bromo benzylamine, ethyl glyoxalate and aryl/heteroaryl boronic acids. The reaction in the presence of CO or allenes resulted in isoindolone- or 4-methylene-3,4-dihydroisoquinoline α-amino acid (Scheme 1) derivatives, respectively in good yield.[5]

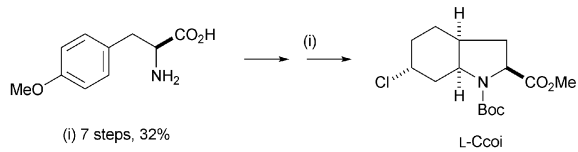
A pyrroline nitroxide based cyclic tetrasubstituted α-amino acid and paramagnetic homoPro and their derivatives have been described.[6] Introduction of a new paramagnetic protecting group to follow the incorporation of an amino acid into peptides is also suggested.

Aeruginosins constitute a new group of structurally related cyanobacterial peptides, bearing a C-6 functionalized *cis*-octahydroindole-2-carboxylic acid derivative as their common structural feature. An efficient and stereoselective route

[a] *University of Szeged, Department of Medical Chemistry, Dom ter 8, Szeged H-6720, Hungary*



Scheme 1

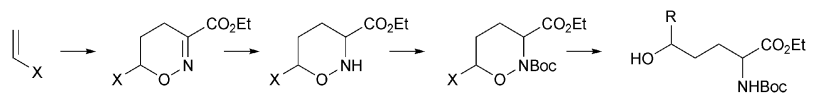


Scheme 2

to a new α-amino acid has been reported (Scheme 2), which is the proposed core of aeruginosins 205.[7]

New bicyclic acidic amino acids, which are conformationally constrained homologues of Glu, were prepared *via* a strategy based on a 1,3-dipolar cycloaddition.[8]

The increasing interest of modified peptides in the chemical engineering of proteins and also as therapeutic agents has refreshed research towards the development of new methodologies for the stereoselective production of natural and unnatural α-amino acids. A new method for the stereoselective synthesis of protected nonproteinogenic α-amino acids, having the structure of a branched bis-homoSer (Scheme 3) or proline can be prepared stereoselectively by **hetero-Diels–Alder addition** of ethyl 2-nitrosoacrylate to electron-rich alkenes, such as enol ethers and allylsilanes, and a further two or three step manipulation of the resulting oxazine.[9]



Scheme 3

2.3 Miscellaneous

Over the last 25 years, significant efforts have been devoted to the development of polymeric biomaterials. These materials have to be biologically inert and stable under physiological conditions. Since polyamino acids are structurally related to natural proteins, the synthesis of amino-acid-based polymers was explored as a potential source of new biomaterials. The phenolic hydroxyl group of the natural amino acid L-Tyr makes it possible to use derivatives of Tyr dipeptide as a motif to generate diphenolic monomers, which are important building blocks for the design of biodegradable polymers. Particularly useful monomers are desaminotyrosyl-Tyr alkyl esters. Using this approach, a wide variety of polymers (tyrosine-derived polycarbonates, polyarylates, and polyethers) have been synthesized and are reviewed with special emphasis on recent developments.[10]

In the past 20 years, a large number of compounds have been developed as β-turn mimics, but previously, there was no general method for keeping all the side chain functions of the natural β-turns, which limits the ability of the motifs to mimic natural turns. The 3-substituted prolines and particularly the *cis* isomers with the

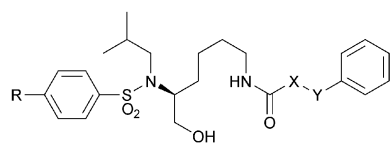
appropriate side chain can be used to mimic type I, II or II′ β-turns and incorporate the side chain functionalities on both the $i + 1$ and $i + 2$ positions of β-turns.[11]

A number of experimental evidences suggested that acivicin [(αS,5S)-α-amino-3-chloro-4,5-dihydro-isoxazol-5-yl acetic acid] could be potentially useful in the treatment of certain tumors, *i.e.* myeloid leukemia. However, a number of severe side effects associated with the use of acivicin emerged during clinical trials. Therefore, acivicin represents an excellent lead for the design of new, structurally related amino acid derivatives with an improved selectivity and a reduced toxicity. A set of conformationally constrained analogues of acivicin has been designed and synthesized.[12]

In α-cyclodextrin six D-glucose units are covalently linked to form a torus shaped molecule with a rigid cavity. Cucurbit[6]uril is a highly symmetric molecule like α-cyclodextrin. It is formed from urea, glyoxal, and formaldehyde during an acid-catalysed reaction. Six glycoluril units form a rigid molecule with a cavity. Both the natural and the synthetic ligand are closely related and are able to enclose a large number of guest molecules within their cavities. The complex stabilities and the thermodynamic data for the complexation of α-cyclodextrin and cucurbit[6]uril with some amino acids (Gly, L-Ala, L-Val, L-Phe, 6-amino hexanoic acid, 8-amino octanoic acid, 11-amino undecanoic acid) and dipeptides have been determined in aqueous solution by calorimetric titrations. The stabilities of the complexes formed are of the same order of magnitude. In case of α-cyclodextrin, the hydrophobic interactions are responsible for the observed values. For cucurbit[6]uril, ion–dipole interactions are the important factor.[13]

Nonnatural amino acid mutagenesis makes possible the site-specific incorporation of synthetic amino acids and allows detailed studies of ion channels with admirable molecular precision in a cellular environment. The ability to incorporate synthetic amino acids allows systematic structure-function studies, furnishing a chemical-scale precision at the level of single atoms and bonds. The methodology permits study in a cellular system, allowing direct and relevant functional analysis of the mutated channels.[14]

One of the main strategies applied to address the AIDS epidemic consists of the inhibition of the virally encoded enzyme human immunodeficiency virus (HIV) aspartyl protease. Simple and potent anti-HIV protease compounds were fashioned from the amino acid L-lysine. A series of N^{α}-isobutyl-N^{α}-arylsulfonamido-(N^{ε}-acyl) Lys and lysinol (**1**) derivatives were prepared and evaluated as inhibitors of HIV protease and wild type virus. A simple original synthesis was devised to form N^{α}-(arylsulfonamide)-N^{α}-isobutyl Lys, which could be easily acylated with carboxylic acids at the N^{ε} position. A two-atom spacer was found to be optimal between this acyl group and a phenyl yielding compounds of sub-nanomolar potency on purified enzyme.[15]



(**1**)

The synthesis of ε-Poly-L-Lys (ε-PL), a unique homopolypeptide, has been investigated in a cell-free system.[16] It has been suggested that its synthesis is similar to that of poly-(γ-D-Glu) in terms of adenylation of the substrate amino acid.

More than 30 novel amino acids have been genetically encoded in response to unique triplet and quadruplet codons including fluorescent, photoreactive and redox active amino acids, glycosylated and heavy atom derived amino acids in addition to those with keto, azido and acetylene chains.[17]

3. Chemical synthesis and resolution of amino acids

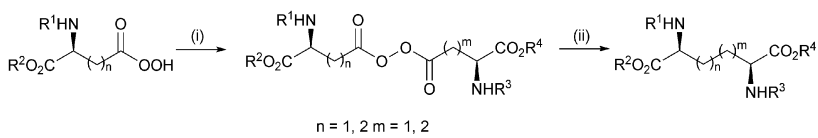
3.1 General methods

Benzyloxycarbonyl (*Z*) group is a widely utilized amine protecting group in peptide chemistry. Hydrogen in the presence of Pd/C catalyst is often used for its removal. An alternate method for the removal of the Z group involves the use of acids, such as trifluoroacetic acid/thioanisole; however, this method can potentially damage other acid-labile groups. In view of the current interest in environmentally benign organic synthetic methodologies, much attention has been paid to the development of highly efficient heterogeneous catalysts for removal of the *Z* group with functional group tolerance. Hydroxyapatite-bound Pd catalyst was found to be highly effective for the deprotection of the *Z* group from amino acids in the presence of molecular hydrogen. The catalyst was also applicable to the hydrogenolysis of a sterically encumbered core-*Z*-protected poly(amido amine) dendrimer.[18] Fluorous carbobenzyloxy ((F)Cbz) reagents $RfCH_2CH_2C_6H_4CH_2OC(O)OSu$ (where Su is succinimidoyl and Rf is C_6F_{13} and C_8F_{17}) have been used to make (F)Cbz derivatives of 18 of the 20 natural amino acids.[19]

The formyl protecting group in combination with a *tert*-butyl ester group is useful in preparing highly functionalized peptide derivatives, but standard *N*-formylation is incompatible with *tert*-butyl groups. A simple and useful methodology has been developed for the preparation of *N*-formyl amino acid esters using the inexpensive, readily available, and environmentally acceptable reagent ammonium formate. Amino acid ester hydrochlorides were reacted with ammonium formate to give *N*-formyl amino acid esters.[20]

The synthesis of mixed anhydrides of polyamino-polycarboxylic acids, amino acids, and phosphoric acid was presented. The obtained compounds were effective as flame retardants at low concentrations where classical fire retardants are not effective.[21]

Photolysis of the amino acid derived symmetrical and unsymmetrical diacyl peroxides at 254 nm at low temperature (−78 to −196 °C) generates various bis(amino acids) in a concise manner (Scheme 4) and with orthogonal protection. The methodology was applied to the synthesis of (4R)-5-propyl-L-Leu.[22]



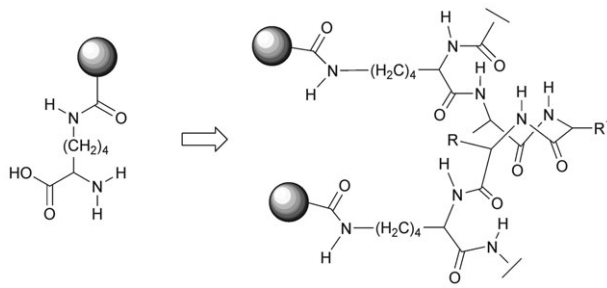
Scheme 4

Synthesis of Fmoc-/Boc-/*Z*-β-amino acids *via* **Arndt-Eistert homologation** of Fmoc-/Boc-/*Z*-α-amino acids employing BOP and PyBOP as a coupling agent to the corresponding β-amino acids and synthesizing the key intermediate α-diazoketones as crystalline solids in good yield were described.[23] The synthesis of optically active amino acid over Pd catalysts impregnated on mesoporous support has been described. These catalysts afford a high level of enantio-selectivity in the asymmetric hydrogenation of α-keto acids to corresponding amino acids.[24] A series of new 4-[2′-(6′-nitro)benzimidazolyl]benzoyl amino acids and peptides have been synthesized by coupling the 4-[2′-(6′-nitro)benzimidazolyl]benzoic acid with amino acid methyl esters/dipeptides using DCC as the coupling agent.[25]

Although solid-supported reagents and scavengers have been used in organic synthesis for decades, it was the development of combinatorial and parallel high throughput synthesis techniques that brought this class of reagents to wider

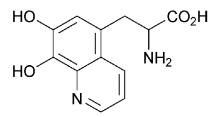
attention. A convenient and general procedure is described with the application of the polystyrylsulfonyl-3-nitro-1*H*-1,2,4-triazolide-resin which is readily available from the corresponding commercially available polystyryl sulfonyl chloride resin. The new resin was used for the formation of esters of Fmoc-protected α-amino acids in high yields and purity with a low level of racemisation. When compared to polystyryl sulfonyl chloride resin, the new solid-supported reagent reduces considerably the amount of racemisation.[26] All by-products can be removed by simple filtration and extraction work-up without the need for chromatography.

Among the new challenges of chemistry are macromolecular entities composed of many identical components, arranged to serve as receptors for given binding units or ligands. Multifunctionality of receptor molecules reflects a current trend towards 'smart' materials, informationally rich molecular devices, and nanofabrication. A modular strategy towards receptor macromolecules is presented, which combines synthetically diverse peptide synthesis with highly functional calixarene chemistry. The design and synthesis of calix[4]arene amino acids (calix-lysines) are described (Scheme 5), which were used as construction blocks to assemble nanoscale, multivalent entities—calix-peptides and calix-peptide-dendrimers.[27]



Scheme 5

3-(7,8-dihydroxyquinolin-5-yl)-L-Ala (**2**) can be expected to form metal complexes. In addition, it can participate in electron transfer reactions and may be regarded as a biosynthetic precursor of the cytotoxic marine alkaloid halitulin. An efficient synthesis of this interesting amino acid has been described from L-Tyr.[28]



(**2**)

3.2 Asymmetric and stereoselective synthesis

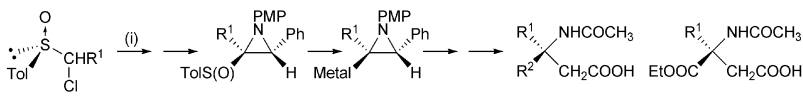
In addition to the 20 natural α-amino acids, there are many other α-amino acids found in various natural products. Although a large number of methods are available for the synthesis of α-amino acids, there is always scope for the development of new and more efficient methods that could be useful for the synthesis of many of the natural and unusual α-amino acids. Broad review of recent developments in the catalytic asymmetric synthesis of α- and β-amino acids has been appeared,[29] and the use of aziridines and oxazolines as valuable intermediates in the synthesis of unusual amino acids has also been summarized.[30]

The catalytic asymmetric addition of organic nucleophiles to α-imino esters has emerged as one of the most promising and intensely investigated routes to optically

enriched α- and β-amino acid derivatives as highlighted in a recent review.[31] Preparation of L-α-amino acids has been easily accomplished simply by exchanging the position of the lactone group of a chiral template from C-2 to C-3. Alkylation of iminolactone afforded the α-monosubstituted products in good yields and excellent diastereoselectivities (>98%). Hydrolysis of the alkylated iminolactones furnished the desired L-α-amino acids in good yields.[32] A high degree of stereocontrol in radical addition to glyoxylic nitrone provided a new method for asymmetric synthesis of α-amino acids.[33]

Since nowadays enantiomeric purity is one of the major issues in α-amino acid synthesis, tremendous efforts have been put into the development of asymmetric versions of Strecker's protocol. Carbocyclic α-amino acids as representatives of the α,α-disubstituted α-amino acid family are widely used in the isosteric replacement of proteinogenic amino acids resulting in specific backbone conformations and increased stability towards chemical and enzymatic degradations. The synthesis of the bicyclic imides as conformationally restricted templates, which mimic folded conformations of Glu has been reported.[34] The application of the asymmetric **Strecker** protocol followed by a ring closure addition-elimination reaction between an amide nitrogen and the ester functionality led to the synthesis of the previously unknown 1*R*, 2*S*- and 1*S*, 2*R*-1-amino-*cis*-3-azabicyclo[4.4.0]decan-2,4-dione hydrochlorides (bicyclic Glu derivatives).[34] The synthesis of a series of optically active α,α—disubstituted α-amino acids has been summarized starting with an achiral or a racemic α-hydroxy or α-diazo ketone. Some problematic processes that remained in the **Strecker synthesis**, *i.e.* preparation of the starting α-acyloxy ketone and oxidative conversion of alpha-amino nitrile into α-imino nitrile, are much improved as regards efficiency by the development of a Cu-catalyzed insertion of α-diazo ketone into N-protected α-amino acid, and as regards yields by the use of ozone as the oxidant. With these methods, various types of α,α-disubstituted α-amino acids have been synthesized including cyclic analogs as a conformational variant of Ser.[35]

Quaternary amino acids (α,α-disubstituted α-amino acids and β,β-disubstituted β-amino acids) have received considerable recent attention in the area of bioorganic chemistry. Several α- and β-amino acid derivatives in enantiomerically pure form has been successfully synthesized from optically active 1-chloroalkyl *p*-tolyl sulfoxides and imine *via* the enantiomerically pure sulfinylaziridine and the aziridinylmagnesium or aziridinyllithium as the key intermediates. From the aziridinyllithium, enantiomerically pure quaternary Phe and quaternary Asp derivatives were synthesized (Scheme 6).[36]

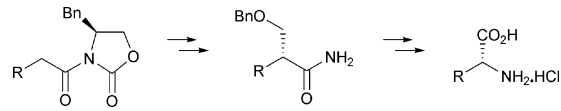


Metal = MgX, Li

(i) PhCH = NPMP

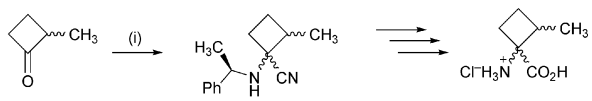
Scheme 6

While the earlier method for the diastereoselective **Strecker** synthesis of α-amino acids using α-phenylglycinol as chiral auxiliary worked well for α-aryl substrates, the selectivities were poor with α-alkyl-substituted compounds. A new method was developed for the stereoselective synthesis of α-amino acids that works equally well with both alkyl and aryl-substituted compounds and can be applied to prepare both D- and L-isomers where the acid functionality was constructed by oxidizing a hydroxymethyl group introduced by Evans' method in the α-position of an appropriate acid substrate and the amino part came from the amide of the original carboxyl group following a modified Hofmann rearrangement reaction (summarized in Scheme 7).[37]



Scheme 7

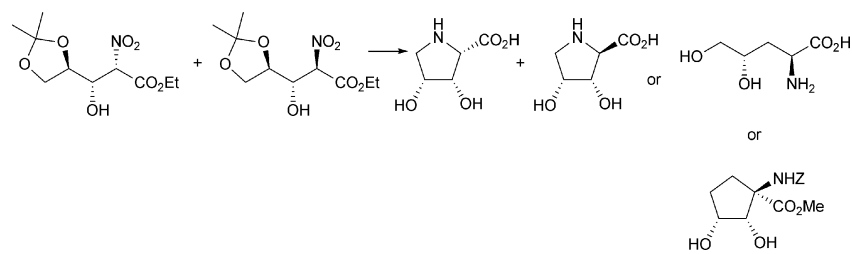
L-Glu is an endogenous ligand for more than 40 glutamate receptors. Since individual glutamate receptors show different distributions within the central nervous system, subtype-selective ligands of these receptors hold promise for treatment of some neurological diseases caused by abnormal activities of glutamate receptors. The **Strecker reaction** of γ-keto acid derived sodium salts with (*S*)-phenylglycinol followed by treatment of the resultant α-amino nitriles with HCl in methanol and heating gave bicyclic lactones. Hydrolysis and subsequent debenzylation and alkylation products furnish α-substituted and α,γ-disubstituted Glu.[38] 1-Aminocyclobutane carboxylic acids have received increasing attention in the field of medicinal chemistry in recent years. The synthesis of enantiopure *cis*- and *trans*-2,4-methanovalines has been achieved by means of asymmetric Strecker synthesis starting from racemic 2-methylcyclobutanone.[39] The *trans*-configured α-amino acids were obtained from cyanide additions carried out in methanol, whereas the *cis*-configured 2,4-methanovalines were accessible *via* reactions in hexane (Scheme 8).



(i) 1. (*R*)-1-PEA, 2. TMSCN; $ZnCl_2$, MeOH and hexane, rsp

Scheme 8

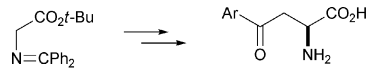
New synthetic applications have been developed for D-glyceraldehyde, which has proved to be a valuable chiral synthon that can be used as an alternative to tartaric, malic and lactic acids in the preparation of optically active naturally occurring compounds. The **Henry reaction** between D-glyceraldehyde and ethyl nitroacetate allowed the practical development of a diastereoselective synthesis of 3,4,5-trihydroxy-2-nitropentanoic acid esters, which were reduced to polyoxamic acids. The latter were used in a new diastereoselective synthesis of 3,4-dihydroxy-Pro and new enantioselective syntheses of D-*threo*-L-norVal and (2*S*,3*R*,4*R*)-2-amino-3,4-dihydroxytetrahydrofuran-2-carboxylic acid methyl ester (Scheme 9).[40]



Scheme 9

Aroyl-Ala derivatives constitute an interesting group of α-amino acids that have attracted considerable interest in recent years as a consequence of studies into the kynurenine pathway, a process that starts with the oxidative cleavage of the essential

amino acid L-Trp to give kynurenine. This pathway appears to play an important role in a variety of fundamental biological processes. The need to study this pathway in more detail, coupled with the recognition that it could offer a means of treating a wide variety of disorders, has led to significant interest in the synthesis of kynurenine and related aroylalanine derivatives. The development of a highly enantioselective method for the synthesis of aroylalanines is described.[41] The approach employs a protected 2-amino-4-bromopent-4-enoic acid, generated *via* the asymmetric phase-transfer catalyzed alkylation of a Gly imine, as a key intermediate. **Suzuki coupling** with an aryl boronic acid followed by ozonolysis of the resulting styrene provides efficient access to the aroyl-Ala derivatives (Scheme 10).



L-Kynurenine (Ar = 2-aminophenyl)

Scheme 10

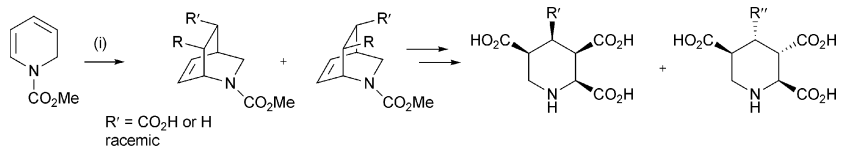
A new asymmetric synthesis of α-amino acids has been described in which the key step is the highly diastereoselective addition of organolithium carboxyl synthons (2-furyllithium, phenyllithium, vinyllithium) to (*R*)- and (*S*)-*O*(1-phenylbutyl) oximes. The method was exemplified by the synthesis of a range of N-protected amino acids and esters and derivatives of non-proteinogenic amino acids such as 4-bromo-Phe, tert-Leu, norVal, cyclohexyl- and aryl-glycines, 2-amino-8-oxodecanoic acid (Aoda) and α-methyl-Val.[42] A new chiral, 2,3-butanedione protected Gly equivalent has been synthesised from glycidol using a chiral memory protocol. Its use in the synthesis of N-Z protected α-amino acids was demonstrated in a series of diastereoselective lithium enolate alkylation reactions and subsequent acid hydrolyses.[43]

Among several enzymatic methods that have been employed for the synthesis of optically active amino acids, transamination is a promising one due to its high turnover number, broad substrate specificity, and no requirement for external cofactor regeneration. Two-liquid phase system has been widely used in biotransformations because it can possibly increase the solubility of immiscible substrate in aqueous media, shifts equilibrium to the direction favorable for products, and alleviates product inhibition at the same time. However, solvent toxicity to enzyme and mass transfer limitation attributed to restricted interfacial area would conversely become drawbacks. An efficient simultaneous synthesis of enantiopure (*S*)-amino acids was achieved using α/ω-aminotransferase coupling reaction with two-liquid phase system. Most of the reactions take place in the aqueous phase, and acetophenone mainly moved to the organic phase according to its partition coefficient. The right solvent based upon biocompatibility and extraction capacity of inhibiting compounds allows a construction of a very simple and easy two-phase reaction system, and it leads to produce enantiomerically pure chiral amino acids at high concentrations. For the simultaneous synthesis of enatiomerically pure (S)-amino acids and (R)-amines from corresponding α-keto acids and racemic amines, an α/ω-transaminase coupled reaction system was designed using favorable reaction equilibrium shift led by the ω-transaminase reaction.[44]

L-Pro and its derivatives have become a series of important molecules in asymmetric catalysis due to its rigid structure, easy availability, and cheapness. The N-terminal protected amino acid (Boc-L-Pro) is a chiral ligand for the enantioselective phenylacetylene addition to aromatic aldehydes under very mild condition, thus expanding the utility of the simplest enzyme, Pro, in asymmetric catalysis. Good yields and enantioselectivities were achieved.[45] An improved synthesis of Pro-derived Ni(II) complexes of Gly has been reported.[46] These versatile chiral equivalents of Gly can be used for general asymmetric synthesis of α-amino

acids. The iridium-catalyzed asymmetric allylic substitution of diphenylimino Gly with allylic phosphates gave β-substituted α-amino acids with high enantioselectivity (up to 97%), when chiral bidentate phosphite bearing the 2-ethylthioethyl group was employed. This method was also applied to the asymmetric synthesis of quaternary α-amino acids.[47]

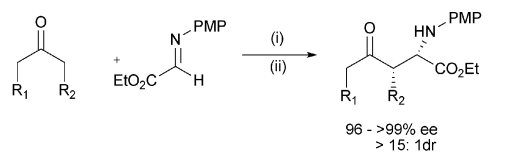
Diels–Alder adducts of 1,2-dihydropyridine with maleic and acrylic acid derivatives were stereospecifically converted by way of RuO_4 oxidation into new piperidine-tetracarboxylic and piperidinetricarboxylic acids (Scheme 11).[48]



Scheme 11

A versatile route to highly regio- and diastereoselective derivatives of α-amino β-hydroxy carboxylic acids, either with tertiary (from a corresponding Gly equivalent) or a quaternary α-carbon center has been described.[49] The key reaction is the cycloaddition of electronically excited carbonyl compounds to oxazoles.

The enantioselective **Mannich**-type reactions of enolates or enolate equivalents with α-imino esters constitutes a powerful approach to the synthesis of novel functionalized γ-keto-α-amino acid derivatives. Over the past few years, catalytic, enantioselective versions of this process has received great attention with a major emphasis being given to the development of organometallic catalysis. The development of metal-free organocatalysts has emerged as a new frontier in asymmetric catalysis. Several catalytic systems including L-Pro,[45] peptides, and small organic molecules have been reported for the Mannich reactions. A pyrrolidine-sulfonamide organocatalyst has been discovered which promotes direct α-aminoxylation reactions of ketones and aldehydes with nitrosobenzene in a highly enantio- and regioselective manner. This novel pyrrolidine-sulfonamide has been prepared and used successfully to catalyze highly efficient, direct, asymmetric Mannich-type reactions between ketones with α-imino esters to produce functionalized α-amino acid derivatives (Scheme 12) with excellent levels of regio-, diastereo-, and enantioselectivity.[50]



Scheme 12

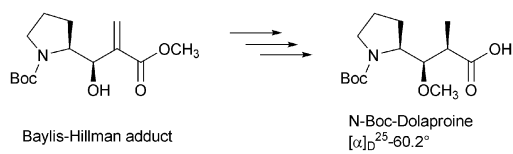
Asymmetric **Mannich**-type reactions has also been used for the preparation of Asp derivatives from homochiral N-tert-butanesulfinylimino esters in diastereomeric ratios up to 97:3. Following an easy removal of the N-tert-butanesulfinyl chiral auxiliary, optically active β-amino esters were obtained.[51]

A method based on the highly diastereoselective **Friedel-Crafts** type reaction of indoles with chiral cyclic glyoxylate imines in the presence of TFA toward the

stereoselective synthesis of 3-indolyl-*N*-substituted Gly derivatives was presented.[52] Optically active α-aryl-Gly derivatives have also been synthesized by this method[53] which has been discussed more detailed in Section 3.4. The stereoselectivity of the ultrasonically induced zinc–copper conjugate addition of iodides to chiral α,β-unsaturated carbonyl systems under aqueous conditions was studied. Alkyl iodides add diastereoselectively to methylenedioxolanone and methyleneoxazolidinone to afford the 1,4-addition products in good yields and with high diastereomeric excess.[54] Since the 1,4-addition products can be readily hydrolyzed, this methodology constitutes a novel entry for the enantioselective synthesis of α- and γ-hydroxy acids and α-amino acids in aqueous media. The results obtained support the radical mechanism and represent one of the few examples of a radical stereoselective conjugate addition in aqueous medium.

While fermentation-based routes have been more economical for the production of the naturally occurring proteinogenic L-amino acids, a niche exists for the use of chemoenzymatic methods in the production of unnatural D- and/or L-amino acids. Enzymatic approaches for the production of amino acids by nitrilases were described.[55] The **Strecker** synthesis of α-aminonitriles, followed by acid- or base-catalyzed nitrile hydrolysis, is one of the oldest and well-known routes to racemic amino acids. Dynamic kinetic asymmetric synthesis conditions were established for the aromatic aminonitriles, phenylglycinonitrile and 4-fluorophenylglycinonitrile, at high pH to produce the corresponding amino acids in high enantiomeric excess (e.e.). *N*-Acylation of aromatic aminonitriles led to spontaneous racemization at pH 8, allowing preferential enzymatic hydrolysis of the (*R*)-enantiomer to afford the product *N*-acylamino acids in up to 99% e.e. Two novel approaches to the chemoenzymatic production of aromatic amino acids have also been described.[55]

α-Hydroxy-β-amino acids were synthesized with excellent yields for the first time in water and by a simple procedure based on a copper catalytic cycle. This is a new protocol for preparation of optically active norstatines.[56] Dolastatin has been reported to exhibit a remarkable antineoplastic activity and is now in Phase II human cancer clinicals trials. The unusual β-methoxy-γ-amino acid dolaproine (Dap) comprises the most complex unit of the pentapeptide dolastatin. The **Baylis–Hillman reaction** between *N*-Boc-prolinal and methyl acrylate, followed by a diastereoselective double bond hydrogenation and hydrolysis of the ester function for the total synthesis of biologically active products resulted in an alternative strategy for the preparation of this important non-proteinogenic amino acid (Scheme 13). This easy and stereoselective synthesis did not require anhydrous solvents, a temperamental boron enolate or a low reaction temperature. Moreover, it was shown that the use of ultrasound radiation significantly decreased the Baylis–Hillman reaction time.[57]



Scheme 13

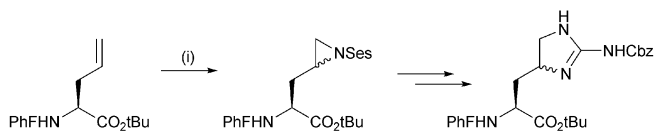
Many of the unnatural amino acids are also critical components in pharmaceuticals and developmental drugs.[58] The synthesis of enantiomerically pure non-proteinogenic α-amino and cyclic amino acids using chiral Ni-II complex and α,α′-dibromo-omicron-xylene as a bifunctional agent of alkylation has been presented.[59] Stoichiometric reduction of the C,N double bond of oxime precursors of α-amino acids was performed in aqueous media by Cr(II) complexes of natural amino acids. The reduction of oximes of α-ketophenylacetic, α-keto-β-phenylpropionic, and

α-ketopropionic acids proceeded up to >90% conversion. Complexes of Cr(II) with L-Ala, L-Val, L-Asp, L-His and L-Phe (1:2) were used as reducing agents.[60]

A new enantioselective synthesis of furan-2-yl amines and amino acids has been described, in which the key step is the oxazaborolidine-catalyzed enantioselective reduction of O-benzyl (*E*)- and (*Z*)-furan-2-yl ketone oximes to the corresponding chiral amines. Oxidation of the furan ring furnished amino acids in high yields.[61] The asymmetric synthesis of α-amino acids under phase-transfer conditions has been widely studied over the last 15 years, leading to very high enantioselectivity. New chiral poly(ethylene glycol) bound *Cinchona* alkaloid salts with two different anchorage sites, and two different chain lengths have been synthesized. Application to the enantioselective alkylation of Gly Schiff base under phase-transfer conditions has clearly demonstrated high catalyst efficiency in terms of reaction time and yield, ee's are strongly dependent on the nature of the alkaloid, anchorage site, and linear polymer length.[62]

A novel synthetic method has been reported to convert simple β-keto esters into enantiomerically enriched α-amino acids. The key features of this process include the addition of azide to the C3 position of β-keto ester derived N-tosyloxy-beta-lactams through a concomitant nucleophilic addition, N–O bond reduction reaction, a mild CsF-induced N1 benzylation of α-azido monocyclic beta-lactams, the preparation of α-keto-beta-lactams through a novel four-step sequence from the corresponding 3-azido-1-benzyl-beta-lactams, and TEMPO-mediated ring expansion of these compounds to the corresponding N-carboxy anhydrides (NCAs).[63] The N-tosyloxy-beta-lactams have also been used for the enantioselective synthesis of protected erythro-α,β-diamino acids. The reported approach is flexible and compatible with a variety of functional groups.[64]

Rigid ligands can lead to greater lipophilicity and/or increased stability toward metabolic enzymes, both factors contributing to improved bioavailability of a given active substance. The synthesis of fully protected aminodihydrohistidines in optically pure form has been described starting from allyl-Gly derivatives. These compounds represent novel conformationally constrained analogues of Arg. The key step of the strategy is a one-pot copper-catalyzed iminoiodane-mediated aziridination of *t*-butyl (*S*)-*N*-(9-phenyl-9*H*-fluoren-9-yl)allyl-Gly with 2-trimethylsilylethanesulfonamide($SesNH_2$) in the presence of iodosylbenzene. This method was used for the preparation of novel rigid analogues of Arg (Scheme 14) starting from the stereoisomeric aziridinated products.[65]



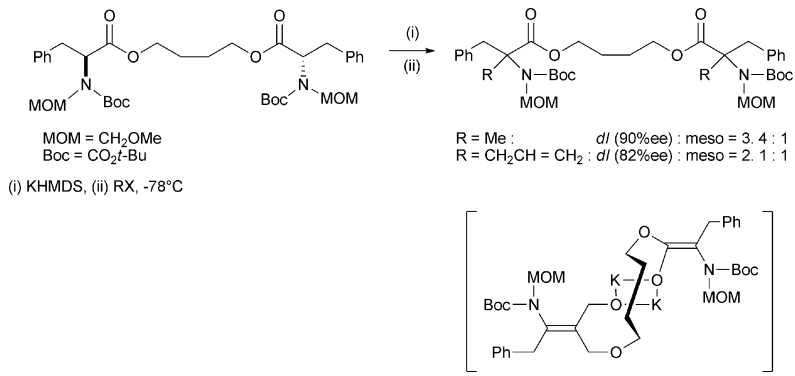
(i) $SesNH_2$, PhI = O, $Cu(CH_3CN)_4PF_6$

Scheme 14

The availability of synthetic methods allowing the design and synthesis of tailor-made amino acids and related compounds is a critical component of any current effort to understand the proteome and its relation to life, health, and disease. In particular, considerable effort has been focused on developing conformationally constrained analogs of aromatic amino acids because of their importance in protein folding and recognition. It has been demonstrated that the readily available amido–keto compounds, with prearranged carbonyl and Gly moieties, under strongly basic conditions easily undergo complete and highly diastereoselective

cyclization, affording a generalized and practical access to the conformationally constrained phenyl-Ser derivatives. High chemical yields, virtually complete diastereoselectivity combined with the operational convenience of the experimental procedures render this method useful for preparation of these diastereomerically pure derivatives.[66]

Two strategies were introduced for the control of enantioselectivity of alkylation of Phe derivatives by regulation of the aggregate structure of chiral enolate intermediates. The use of amino acid-dimers, was effective to minimize solvent- and electrophile-dependency of enantioselectivity of the alkylation. α-Allylation proceeded in improved selectivity of 82–88% ee under the control of aggregation of the enolate intermediate (Scheme 15).[67]

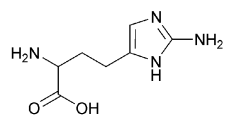


Scheme 15

3.3 Synthesis of naturally occuring and nonnatural α-amino acids

Compounds capable of inhibiting Gly transport function could provide various therapeutic benefits for a number of disorders associated with excessive neuronal activity. Therefore, molecular entities that are capable of blocking the Gly reuptake process should also be effective in enhancing inhibitory activity. A variety of α-amino acid derivatives were prepared as Gly transport inhibitors.[68,69] Substituents at the chiral center was studied and L-Phe was identified as the preferred amino acid residue. Compounds prepared from L-amino acids were more potent GlyT-2 inhibitors than analogs derived from the corresponding D-amino acids.[69]

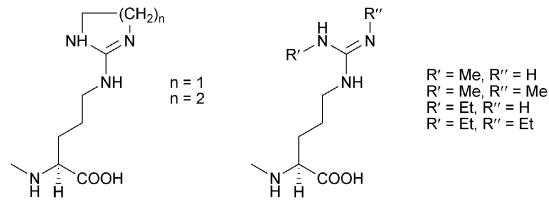
The guanidinium group of L-Arg is involved in many physiological and patho-physiological processes. Interactions with the guanidinium moiety are mediated by the carboxylic groups of the side chains of Asp or Glu. A convenient synthetic access to the unknown amino acid L-α-aminohomo-His (L-Ahh) has been developed starting from the readily available δ-hydroxy-L-Lys. The embedding of the basic guanidino moiety in the aromatic imidazole lowers the basicity of the side chain to a pK_a of 8.3. It is proposed that L-Ahh may be employed as an Arg-mimetic **(3)** in medicinal chemistry.[70]



(3)

A series of N^{α}-methyl-N^{ω}-alkyl-L-Arg analogues **(4)** have been synthesized from inexpensive, commercially available starting materials. These analogues are expected

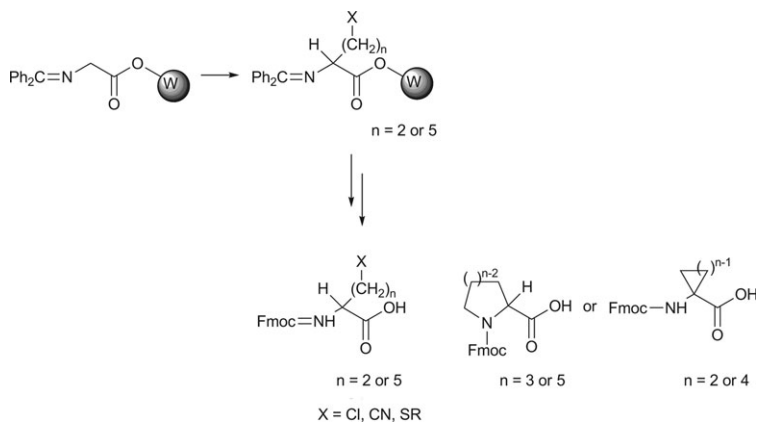
to increase binding affinity, receptor selectivity, lipophilicity, and stability as demonstrated with analogues of similar design and structure.[71]



(4)

Enantiopure (3*S*, 5*R*, 8*S*)-3-[*N*-(Boc)amino]-1-azabicyclo[3.3.0]octan-2-one 8-carboxylic acid was synthesized in nine steps from Asp beta-aldehyde. Since pyrrolizidinone amino acids can serve as conformationally rigid dipeptide surrogates, this synthesis should facilitate its application in the exploration of conformation-activity relationships of various biologically active peptides.[72]

Unique conformational constraints can also be induced into peptides or peptidomimetics by introducing an appropriate amino acid precursor. Unnatural amino acids are generally prepared by synthetic routes involving solution-phase techniques, and subsequently incorporated into a peptide sequence by solid-phase methods. Alkylation of the benzophenone imine of Gly-Wang resin with α,ω-dihaloalkanes yielded valuable reactive intermediates. These racemic ω-chloro or ω-bromo intermediates were converted on-resin to α-amino acids containing diverse side-chain functionalities (*e.g.* ω-chlorides, nitriles, and thioethers), Pro and its ring homologs, and 1-aminocycloalkanecarboxylic acid derivatives (Scheme 16).[73] The incorporation of the ω-haloalkanes into peptides could provide potential alkylating agents for applications such as affinity labeling and intramolecular cyclization.



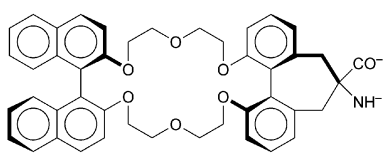
Scheme 16

Efficient syntheses of L-homoisoSer and D,L-homoisoCys derivatives starting from L-malic and D,L-thiomalic acid using hexafluoroacetone as protecting and activating agent have been described.[74] The new compounds are interesting building blocks for the preparation of non-natural peptides and depsipeptides as well as for the construction of new GABA derivatives.

3.4 α-Substituted analogues of amino acids

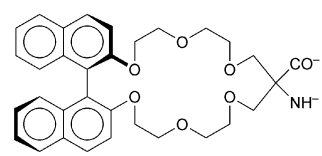
Optically active α,α-disubstituted α-amino acids have generated ever-increasing interest in biology and pharmacology as well as in chemistry. The inclusion of α,α-disubstituted α-amino acids in a peptide may affect its secondary or tertiary structure. α,α-Dimethylglycine (Aib) is the most available and studied member of this type of sterically constrained amino acids. Amino acids other than Aib are not readily available, and, therefore, their biological properties and applications, as sterically constrained scaffolds, are still awaiting systematic studies. It was demonstrated that the readily available Ni(II)-complex derived from Gly Schiff base with 2-[*N*-(α-picolyl)amino]benzophenone easily undergoes complete bis-alkylation with various alkyl halides, and in particular iodides resulting in a practically useful method for preparing symmetrically α,α-disubstituted α-amino acids.[75] This method was systematically studied as a general method for preparing symmetrically α,α-disubstituted α-amino acids and was shown to be particularly successful for the dialkylation of the complex with activated and nonactivated alkyl halides, including propargyl derivatives.[76] This study has also shown some limitation of the method, as it cannot be extended to α- or β-branched alkyl halides or Michael acceptors to be used for the dialkylation reaction. The syntheses of various types of optically active α,α-disubstituted α-amino acids using asymmetric Srecker route have been summarized.[35] For producing chiral nonracemic α,α-dialkylated amino acids, an extension of the methodology which creates quaternary carbon centers of high optical purity using an S_N2' dispalcement on pivalate esters was reported.[77]

Considering the well-documented high tendency of α-amino acids disubstituted at their α-carbon to induce ß-bends and $\alpha/3_{10}$-helical secondary structures in polypeptides, a new series of $C^{\alpha,\alpha}$-disubstituted glycines bearing binaphthol-based crown-ethers: [(*R*)-Binol-22-C-6]-(*R*)-Bip (**5**), [(*R*)-Binol-22-C-6]-(*S*)-Bip, [(*R*)-Binol-21-C-6]-Hms (**6**) and [(*R*)-Binol-20-C-6]-(*L*)-Mdp (**7**), have been synthesized.[78] The chirality of the binaphthyl unit could function in synergy with the chirality of the peptide chain in new crown or poly-crown catalysts with enhanced chiral recognition properties.



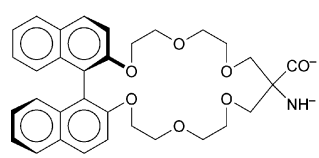
[(*R*)-Binol-22-C-6]-(*R*)-Bip

(**5**)



[(*R*)-Binol-21-C-6]-(*R*)-Hms

(**6**)



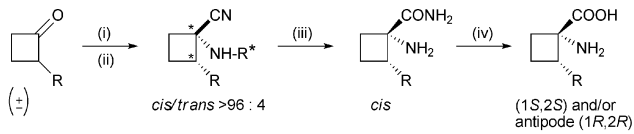
[(*R*)-Binol-20-C-6]-(*L*)-Mdp

(**7**)

α,α-Disubstituted derivatives of Asp are interesting subjects for study because of their relevant role in physiological events and in the stabilization of reverse turns through interactions between backbone NH and side chain $C^{\gamma}O$ bonds. The controlled opening of the N1–C2 bond in 1-carbamate-substituted 2-azetidinones derived from amino acids by O- and N-nucleophiles provides a straightforward access to orthogonally protected α-alkyl Asp and Asn derivatives. The feasibility of

the Phe-derived β-lactams opening to produce orthogonally protected α-benzyl Asp and Asn derivatives could be extended to other α-alkyl analogues, just by using 2-azetidinones derived from other amino acids as starting materials. The procedures described could have a widespread application for the generation of α-alkyl Asp/Asn derivatives in enantiomerically pure form.[79]

It has been shown that a copper complex is capable of catalysing the asymmetric alkylation of enolates of a range of amino acids under very mild reaction conditions, thus allowing the synthesis of enantiomerically enriched α,α-disubstituted amino acids. There is a clear relationship between the size of the side chain of the amino ester substrate and the enantioselectivity of the process.[80] An efficient and easy one-pot reaction from readily available racemic 2-substituted cyclobutanones gave two major aminonitriles with excellent diastereoselectivity. After separation, the major *cis*-aminonitriles were hydrolysed and hydrogenolysed to lead for the first time to pure non-racemic (+)-1-amino-2-isopropylcyclobutanecarboxylic acid and its antipode. In the presence of sodium cyanide and a chiral amine, the racemic α-alkylcyclobutanones underwent a one-pot asymmetric **Strecker** reaction to give the corresponding amino nitriles with high diastereoselectivity. After separation, the resulting amides furnish new enantiopure 1-amino-2-isopropylcyclobutanecarboxylic acids (Scheme 17) in two steps.[81]

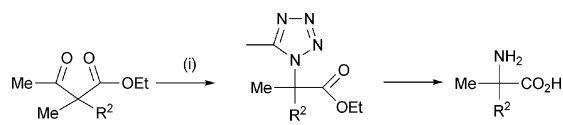


(i) H_2N-R*, NaCN, DMSO, (ii) AcOH, 55-60°C, 4 d, 42-57%, (iii) 1. separation, 2. cc. H_2SO_4, 3. H_2 70-90%
(iv) 1. HCl 6M, 2. methyl epoxide, 90-94%

Scheme 17

A new simple and convenient method has been developed for the synthesis of tetrazole precursors of α-dialkylated α-amino acids (Scheme 18), using trimethylsilyl azide with various α-dialkylated β-keto esters. Quaternization of the tetrazole heterocycle with methyliodide, followed by basic hydrolysis with concentrated KOH affords the α-azido acid, direct precursor of the α-dialkylated α-amino acids.[82]

A large laboratory scale synthesis of (*S*)-α-methyl-Phe from benzaldehyde and



(i) $TMSN_3$ 2.5 eq, $ZnBr_2$ 1 eq, 60 °C,18 h

Scheme 18

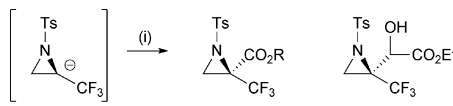
methyl cyanoacetate has been developed. The synthesis is based on the preparation of racemic 2-cyano-2-methyl-3-phenylpropanoic acid, resolution of the enantiomers, and development of an efficient enantioconvergent synthesis of (*S*)-α-methylPhe from enantiopure (*S*)- and (*R*)-2-cyano-2-methyl-3-phenylpropanoic acid. The use of simple and mild reaction conditions that avoid difficult purification procedures and inexpensive and readily available reagents, as well as the high overall yield make this synthetic method very attractive.[83] A similarly efficient synthesis of (*S*)-α-benzyl-α-methyl-β-Ala from benzaldehyde and methyl cyanoacetate has also been developed. The synthesis avoids low temperature reactions and difficult purifications, thus making it amenable to large-scale synthesis.[84] Enantiocontrolled synthesis of

α-methyl amino acids proceeds *via* the regioselective organocuprate opening of Bn-*N*-2-α-methylSer-β-lactone. From this chiral intermediate, a wide variety of α-methyl amino acids and building blocks were synthesized in excellent yields.[85]

A new method was developed for the stereoselective synthesis of α-substituted Ser analogues. A common enantiomerically enriched intermediate was obtained through an enzymatic desymmetrization. A variety of amino acids were synthesized in good ee's through nucleophilic acetylide addition reactions and Pd-catalyzed Sonogashira couplings.[86] Alkylation of the enolate of the Seebach (*R*)-Met oxazolidinone with benzyl bromide gave the expected α-benzylated product in low yield. The major product was a novel amine arising from oxazolidinone cleavage, decarboxylation, alkylation and hydrolysis.[87] The rearrangement could be suppressed by using a more reactive electrophile or the N-Z instead of the N-benzoyl protecting group, and the required (*R*)-α-benzyl-Met was obtained in 78% yield and in an enantiomeric ratio 90:10.

Arylglycines constitute an important class of non-proteinogenic α-amino acids. Unlike other amino acids, which can be effectively prepared in enantiomerically pure form, the apparent simplicity of the aryl-Gly structure is complicated by the ease of racemization at the α-stereocenter. Optically active α-aryl-Gly derivatives were synthesized by Brønsted acid (TFA)-promoted Friedel–Crafts reaction of various phenols with chiral cyclic glyoxylate imines, followed by deprotection with $Pd(OH)_2$/C under H_2. The diastereoselectivities of the initially formed F–C reaction products are up to 99%. The method would allow the syntheses of a variety of highly functionalized and optically pure aryl-Gly derivatives.[53]

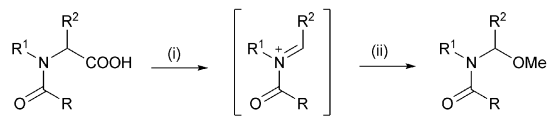
Development of new synthetic methodologies for introduction of fluorine atoms into various bioactive compounds has been eagerly demanded. Among such demands, construction of a chiral trifluoromethylated quaternary carbon center is a tough problem. Optically pure α-trifluoromethylated aziridinyl anions react with various electrophiles to give the corresponding optically pure 2-trifluoromethyl-2-substituted aziridines. The reaction proceeded with retention of the absolute configuration at the trifluoromethylated quaternary carbon center throughout the reactions. The 2-trifluoromethyl-2-substituted aziridines are general synthetic precursors for optically pure α-amino-α-trifluoromethylated compounds, such as trifluoromethylated α/β-amino acids (Scheme 19).[88]



(i) E^+

Scheme 19

Electrochemical oxidation of optically active *N*-acylated α-amino acids gave *N*,*O*-acetals with enantioselectivity. The electrochemical oxidation reactions of *N*-acylated α-amino acids in methanol afford α-methoxylated products with a loss of carbon dioxide through acyliminium ion intermediates (Scheme 20). The nature of



(i) base, $-CO_2$, -2e: (ii) MeOH, $-H^+$

Scheme 20

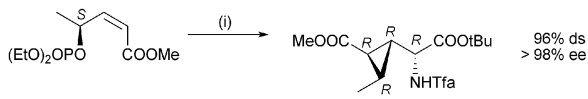
the protecting groups, reaction conditions, and the materials for anode were found to affect the enantioselectivity.[89]

Activation of *N*-terminal resin-bound amino acids as aldimines, followed by alkylation with α,ω-dihaloalkanes, provides key intermediates for the solid-phase preparation of racemic α-substituted Pro ring homologues bearing amino acid side chains. Two intramolecular displacement strategies, one involving an amine nucleophile, the other an amide nucleophile, were used to convert the intermediate ω-chloro or ω-bromo derivatives to the desired cyclic products. Using one of these routes and a fully automated synthesizer, a 48-membered library of α-substituted Pro was prepared.[90]

Convenient asymmetric[91] and solid-phase[92] syntheses of α,α-difluoro-β-amino acids were carried out by Reformatsky reaction.

3.5 Aliphatic and cycloaliphatic α-amino acids

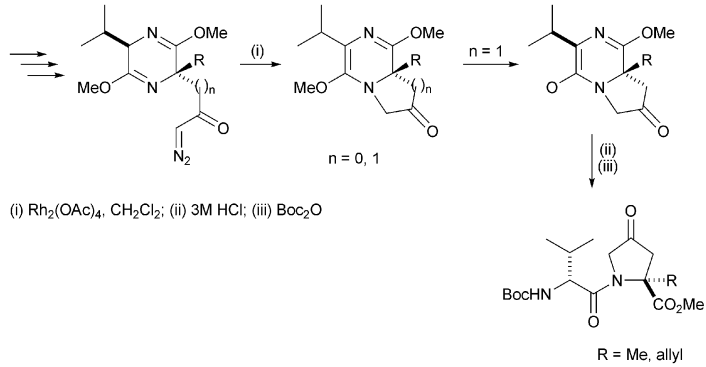
Cyclic amino acids such as carboxycyclopropylglycines have attracted interest due to their remarkable biological activity. They can be regarded as conformationally fixed analogues of Glu. It has been shown that these amino acids can interact very strongly and selectively with the metabotropic glutamate receptors in the mammalian central nervous systems. Besides approaches to introduce the amino acid functionality *via* Strecker reaction, several routes have been developed to construct the cyclopropyl unit, *e.g.* by metal-catalyzed cyclopropanations or carbene addition reactions. An approach based on the Michael-induced ring closure concept led to the desired cyclopropylic compounds by using lithium enolates of phenylimino Gly esters as nucleophiles. These Gly synthons react with a wide range of acceptor-substituted unsaturated systems. A highly selective asymmetric approach to (*S*)-[2,2-H-2(2)]-1-aminocyclopropane-1-carboxylic acid has been reported.[93] It has been shown that Zn-chelated amino acid ester enolates are highly efficient nucleophiles for stereoselective **Michael additions**. Subsequent cyclizations provide interesting constrained amino acids (Scheme 21) in high yield and excellent stereoselectivity. Up to four stereogenic centers can be created in one step.[94]



(i) Tfa-GlyOtBu, LHMDS, $ZnCl_2$, 86%

Scheme 21

A synthesis of all four stereoisomers of 1-amino-2-(hydroxymethyl) cyclobutanecarboxylic acid is based on a chiral glycine equivalent employed in both enantiomeric forms. The key step involves the cyclization of the silyl-protected iodohydrins to the corresponding spiro derivatives with the aid of the phosphazenic base.[95] Five- and six-membered cyclic amino acids can be prepared in good yield with high ee *via* tandem rhodium-DuPHOS catalysed asymmetric hydrogenation followed by a rhodium-catalysed hydroformylation cyclisation sequence in a single pot.[96] Methods for the preparation of cyclic rigidified α-quaternary amino acids with the α-carbon embedded in the ring structure has been described.[97] Intramolecular rhodium(II)-catalysed reactions in geminally disubstituted derivatives of the chiron (*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine provide valuable substrates for the preparation of quaternary cyclic α-amino acid derivatives, where both the α-quaternary carbon and the amino nitrogen are to be embedded in a pyrrolidine ring (Scheme 22). Complete chemoselectivity was seen in the rhodium(II) carbenoid reactions. The products are four- and five-membered annulated rings, azetidin-3-one



Scheme 22

and pyrrolidin-3-one derivatives. The five-membered products are stable and can be hydrolysed to α-quaternary derivatives of Pro.[97]

A new constrained bicyclic Pro-mimetic was developed. The synthesis was achieved by starting from appropriate stereoisomers of Ser and α,β-isopropylidene-glycerol derivatives, thus allowing the preparation of analogues of either L- or D-Pro. The scaffolds were prepared as *N*-Fmoc-amino acids suitable for solid-phase peptide synthesis.[98]

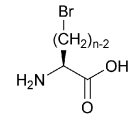
One common strategy for rigidification of analogues of natural amino acids has been to incorporate one or two rings into the α-amino acid. Fused bicyclic α-amino acids can be prepared by a double Michael reaction of *p*-anisyl ethynyl ketone and a tethered diacid, cyclization *via* hydrogenation or hydration of a CN group, and oxidation of the *p*-anisyl group. Bicyclic α-amino acids prepared in this way included *cis*- and *trans*-perhydroisoquinoline-3-carboxylic acids and *cis*-perhydro-2-pyrindine-3-carboxylic acids of various substitutions and oxidation levels. The bicyclic α-amino acids may be regarded as functionalized and conformationally restricted analogues of Pro, pipecolic acid, 2-aminoadipic acid, or Glu.[99]

3.6 Halogenoalkyl α-amino acids

The incorporation of fluoride can have dramatic effects on peptide stability and protein–protein interactions. β-Fluorinated amino acids have gained prominence as mechanism-based inhibitors of amino acid decarboxylases and transaminases. C^{α}-Fluoroalkyl substituted amino acids bearing a fluorinated substituent instead of the α-proton are known to be able to increase metabolic stability as well as stabilize the secondary structure of the peptide. The high lipophilicity of fluoroalkyl substituents has a positive effect on transport properties and *in vivo* absorption of peptides. Due to the high electron density, peptides containing a fluorinated alkyl substituent are capable of interacting with enzyme or receptor subsites in a manner, which is impossible for the fluorine-free pendants. Most synthetic routes to enantiomerically pure C^{α}-fluoroalkyl substituted amino acids rely on chemical and enzymatic resolution. Racemic C^{α}-fluoroalkyl amino acids have been synthesized from fluorinated pyruvates. A method for the enzymatic resolution of racemic C^{α}-fluoroalkyl amino acid amides has also been developed.[100]

Optically pure aliphatic amino acids having easily replaceable functional groups at the ω-positions are highly interesting synthetic targets, as the functional group at the ω-position can be modified to the required group, which provide a series of non-natural amino acids. The scope of the reported methods for the synthesis of ω-halo-α-amino acids are limited due to many reasons such as, the formation of racemic products with difficulty in purification, involve tedious procedures and resulting in

low yields, and could not use 9-fluorenylmethoxycarbonyl (Fmoc) protection strategy. L-α-amino-ω-bromoalkanoic acids (**8**) with side chain lengths varying from 4 to 10 methylene units have been conveniently synthesized as useful intermediates for the synthesis of functionalized non-natural amino acids.[101] The *N*-terminals of these amino acids can be easily protected with Boc or Fmoc groups quantitatively.



Abn (n = 6, 7, 8 ect.)

(**8**)

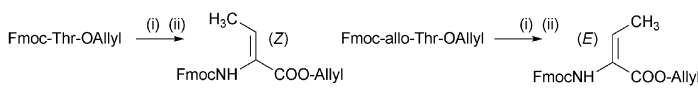
3.7 Hydroxy α-amino acids

A new photoaldol route to α-amino-β-hydroxy carboxylic acid esters is initiated by the photocycloaddition of aromatic or aliphatic aldehydes to 5-methoxyoxazoles. The 4-unsubstituted 5-methyloxazole gave the cycloadducts in high yields and excellent exo-diastereoselectivities. Hydrolysis of the cycloadducts gave the N-acetyl α-amino-β-hydroxy esters, which could be subsequently converted into the corresponding *Z*-didehydro α-amino acids.[102] Aldol reactions using bis-(chiral α-methylbenzyl)Gly esters with aldehydes gave excellent diastereoselectivity. This method was extended for formation of β-hydroxyamino esters.[103]

Synthesis of orthogonally protected (2*S*, 4*R*)- and (2*S*, 4*S*)-4-hydroxyornithine was reported featuring an asymmetric alkylation of *N*-(diphenylmethylene)Gly tert-butyl ester by (5*S*)-*N*-benzyloxycarbonyl-5-iodomethyl oxazolidine. Double stereoselection was examined using chiral ammonium salts as phase transfer catalysts, and a substrate-directed chiral induction is documented.[104]

3.8 α-Amino acids with unsaturated side chain

Dehydropeptides containing α,β-unsaturated amino acid residues are frequently found in natural resources with important biological activity. Their structures are rigid in both the backbones and the side chains of the peptides because of the presence of a double bond conjugated with a peptide linkage. In addition, dehydropeptides are known as fairly reactive Michael acceptors that react readily with 'soft' nucleophiles, such as thiols or amines of biological molecules. This reactivity is thought to be one of the molecular mechanisms underlying the biological activities of dehydropeptides. A selective synthesis of *Z*- and *E*-ΔAbu from L- and L-*allo*-Thr as starting materials through selenation and oxidative elimination processes with a selenyl linker has been described (Scheme 23). The detailed reaction mechanism of phosphine-assisted selenoether formation is also discussed.[105]



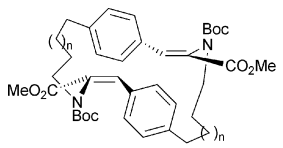
(i) m-tBuOCO-C_6H_4-SeCN, Bu_3P/pyr; (ii) H_2O_2/THF

Scheme 23

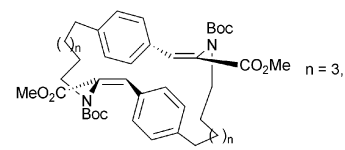
ω-Unsaturated amino acids are of value in terms of their biological importance and their utility as asymmetric synthetic building blocks. The double bond is a masked functional group, and is stable to most acidic and basic reaction conditions. As a precursor, it can be easily transferred to ω-hydroxyl, ω-halogen, ω-epoxy,

ω-amino, aldehyde, and carboxyl amino acids. The enantiomeric syntheses of ω-unsaturated amino acids and β-substituted ω-unsaturated amino acids were accomplished by using the Ni(II)-complex derived from a Gly Schiff base with 2-[*N*-(*N*′-benzylprolyl)amino]benzophenone.[106] It seems to be an efficient, generalized and practically useful method for the large scale preparation of enantiomerically pure ω-unsaturated amino acids.

Examination of the effect of restricting the conformational freedom of a given ligand may lead to increased insight into the bioactive conformation of the ligand and hence ultimately to the generation of more potent and selective molecules. 1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid (Tic) is a conformationally constrained Phe analogue that has been used to considerable effect in medicinal chemistry, its presence in ligands having been shown to influence both affinity and selectivity. The seven-, eight-, nine- and ten-membered analogues of Tic that is Sic, Hic, Nic, and Xic, respectively have been synthesised. The route, based on a new variation of the Heck coupling reaction between iodoarenes and dehydroalanine derivatives, has been used to generate *para*cyclophanes (**9**) and *meta*cyclophanes (**10**) containing unsaturated amino acid residues.[107]



(**9**)



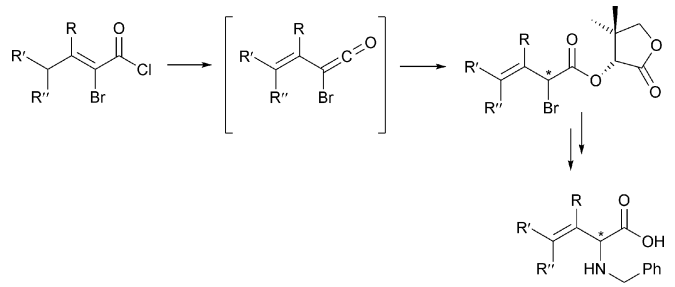
(**10**)

Dehydroalanine amides are important constituents of many natural products. Although the Michael addition is one of the most powerful and widely used synthetic tools, there are only few reports on the Michael addition of nucleophiles to dehydroalanine derivatives since dehydroalanine derivatives are generally very poor Michael acceptors. While most Michael additions are performed in organic solvents, today's environmental concerns encourage the development of "greener" conditions where possible. In water, the rate of Michael addition of amines and thiols to dehydroalanine amides was greatly accelerated, leading to shorter reaction times and higher yields.[108] The ease and efficacy of this method provides an attractive route to the synthesis of natural and unnatural amino acid derivatives from readily available dehydroalanine amides.

Carbon nucleophiles, amines, and oxygen nucleophiles were treated with the methyl ester of *N*-(Boc)-*N*-(p-tolylsulfonyl)-α,β-didehydro-Ala, and also with the methyl esters of *N*-(Boc)-*O*-(p-tolylsulfinyl)-α,β-didehydro-Ser and *N*-(Boc)-β-(1,2,4-triazol-1-yl)-α,β-dide-hydro-Ala, both of which were obtained from the former substrate. Carbon nucleophiles of the β-dicarbonyl type gave furanic amino acids, which were converted into the corresponding pyrrole derivatives (dehydroprolines) in high yields, while use of amines allowed the synthesis of α,α-diamino acids and β-amino-α,β-didehydroamino acids. Different types of alkoxyamino acids were obtained by treatment of the above substrates with oxygen nucleophiles.[109]

Synthetic and naturally occurring β,γ-unsaturated amino acids are known to function as specific enzyme inhibitors of pyridoxal phosphate-dependent enzymes. In addition, it has been demonstrated that the introduction of alkyl, alkenyl or aryl groups in the backbone or at the nitrogen of amino acids, allows the synthesis of conformationally constrained peptides with a rigidified secondary structure and an improved bioactivity and selectivity. The asymmetric synthesis of β,γ-unsaturated α-benzylamino acids has been reported starting from α-bromo-α,β-unsaturated chlorides. The treatment of the acyl chlorides with (*R*)-pantolactone in the presence of TEA, allowed the *in situ* formation of the deconjugated ketenes and their direct transformation into chiral esters. The reactions occurred with good yields and high diastereomeric ratios. The substitution of bromine by benzylamine, followed by acid

hydrolysis, gave enantiomerically enriched α-benzylamino-β,γ-unsaturated acids (Scheme 24). The displacement of the bromine with other nitrogen nucleophiles led to *N*-unsubstituted-β,γ-unsaturated amino acids in good yield with complete diastereoselectivity.[110]



Scheme 24

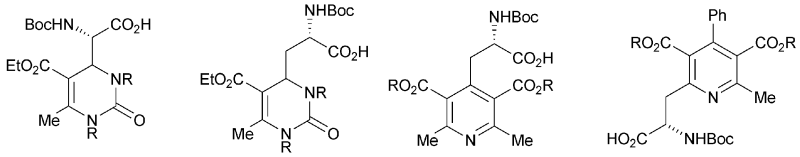
The first catalytic enantioselective method for the synthesis of γ-unsaturated β-amino acids and their corresponding 1,3-amino alcohol derivatives has been presented.[111] This methodology takes advantage of a highly enantioselective vinylzinc addition to an aldehyde to set chirality. The resulting allylic alcohols are then transformed into the corresponding allylic amines *via* Overman's [3,3]-sigmatropic imidate rearrangement, and subsequent one-pot deprotection-oxidation of a pendant oxygen leads to the γ-unsaturated β-amino-acid derivatives of high enantiopurity.

A range of dehydro amino acid derivatives has been prepared and subjected to halogenation. The synthetic utility of the allyl halides prepared in this study is indicated through the synthesis of a cyclopropyl amino acid derivative and the extension of the carbon skeleton of an amino acid side chain.[112] The two enantiomers of derivatives of cyclobutyl-(*Z*)-α,β-dehydro-α-amino acid have been synthesized through respective **Wadsworth–Emmons condensations** of a suitable phosphonate with enantiomeric cyclobutyl aldehydes. These compounds, in turn, were prepared by selective manipulation of the functional groups starting from (–)-*cis*-pinononic acid as the common chiral precursor. These products are suitable for the stereocontrolled synthesis of diferent types of saturated cyclobutyl amino acids and their derivatives.[113]

Attempts to develop anticonvulsive agents in γ-aminobutyric acid (GABA)-related compounds, derivatives of amino acids, and structurally modified compounds of currently used drugs have long been pursued. For the development of new anticonvulsive agents, eight analogs of γ-vinyl GABA has been designed and prepared.[114]

3.9 α-Amino acids with aromatic and heteroaromatic side-chain groups

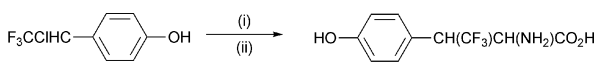
Growing interest has recently been focused on the synthesis of unnatural α-amino acids. The class of nonproteinogenic heterocyclic α-amino acids is of particular interest due to their diverse range of chemical and biomedicinal applications. Most of the developed methods for the synthesis of heterocyclic α-amino acids are based on asymmetric hydrogenation of dehydroamino acid derivatives and coupling reactions of suitable functionalized heterocycles with chiral Gly or Ala equivalents. A novel and versatile strategy for the synthesis of heterocyclic α-amino acids has been described.[115] The use of aldehyde or β-ketoester bearing a masked Gly in Biginelli and Hantzsch cyclocondensations allowed access to the 4-dihydropyrimidinyl-α-glycines (**11**), 4-dihydropyrimidinyl-α-alanines (**12**), 4-pyridyl-α-alanines (**13**), and 2-pyridyl-α-alanines (**14**).



(**11**) (**12**) (**13**) (**14**)

A fast synthesis of ring-A disubstituted Fmoc- and Boc-protected L-Trp analogs was achieved starting from the appropriate 2,4- or 2,3-disubstituted phenylhydrazines and optically active *N*,*N*-diprotected L-Glu γ-aldehydes, utilizing a Fischer-indole synthesis as a key step. Unlike most of the previously reported methods, that required the multistep stereoselective generation of a chiral carbon, this fast methodology is useful for generating optically active ring-A disubstituted protected tryptophans starting from a simple and common chiral precursor.[116]

A novel method using various nucleophiles for the synthesis of β-trifluoromethyl-tyrosine (Scheme 25) has been described.[117]



(i) $O_2NCH_2CO_2Et$, K_2CO_3, DMF; (ii) Pd/C, H_2,CH_3OH

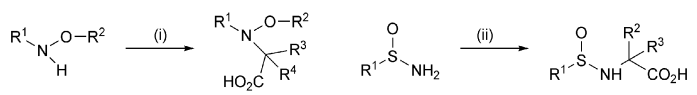
Scheme 25

A facile synthesis of 3-aryl pyroglutamic acids *via* stepwise [3 + 2] annulation and desulfonated hydrolysis is reported. Base-induced coupling/cyclization reactions of α-sulfonylacetamide with various β-functional groups of (*Z*)-2-bromoacrylates yielded three contiguous chiral centers on the polysubstituted pyroglutamates system with *trans–trans* orientation in a one-pot synthesis.[118]

(R)-3-Arylalanines may be prepared in high enantiomeric purity by a four-step reaction sequence involving asymmetric aza-Darzens reaction.[119] An efficient and highly enantioselective method for the preparation of aroyl-Ala derivatives has been developed.[41] The use of amino acid-dimers was effective to minimize solvent- and electrophile-dependency on enantioselectivity of alkylation of Phe derivatives.[67]

3.10 *N*-Substituted α-amino acids

An efficient synthesis of *N*-hydroxy-α-amino acids and derivatives represents a challenging goal in organic synthesis since these compounds are key intermediates in metabolic pathways and can be found in human and animal tumors. *N*-Hydroxy or alkoxy-α-aminocarboxylic acids and *N*-(*tert*-butyl sulfinyl)-α-amino carboxylic acids has been developed from *N*,*O*-alkyl or hydroxylamines and *tert*-butyl sulfinamide utilizing a Petasis boronic acid–Mannich reaction (Scheme 26). The scope and limitations of this method have been examined.[120]



R^1 = Me, Bn, Bu^t, cyclohexyl
R^2 = Me, H
R^3 = aryl, heterocyclic

R^1 = Bu^t
R^2 = aryl, heterocyclic
R^3 = H, Me

(i) $R^3B(OH)_2$, glyoxylic acid, DCM/RT; (ii) R^2CO-CO2H, DCM/RT

Scheme 26

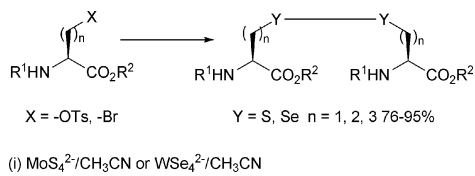
The synthesis and biological evaluation of *N*-substituted alpha-amino acids from 4-hydroxy coumarins have been achieved. A series of *N*-(methylene-4-oxo coumarinyl)carbamates has been produced by the condensation of various carbamates with 4-hydroxy coumarins in the presence of triethylorthoformate.[121]

One way to elaborate α-amino acids and their derivatives is through the use of free radical chemistry, but the principal limitation of this approach is the tendency for hydrogen-atom-transfer reactions of these compounds to afford α-carbon-centered radicals as a result of their stability. Generally this leads to the destruction of the stereochemical integrity of the substrate. *Ab initio* calculations have been used to investigate the effect of *N*-substituents on the stability of α-carbon-centered amino acid radicals. The theoretical and experimental results suggest that *N*-phthaloyl- and triflyl-amino acid derivatives are sufficiently protected against hydrogen-atom abstraction from the α-position.[122]

3.11 Amino acids containing sulphur or selenium

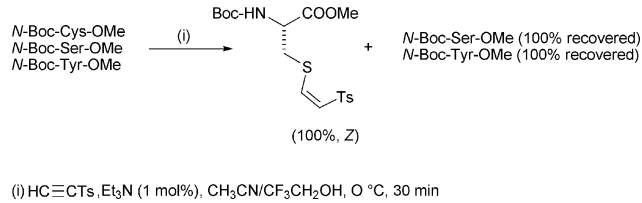
Sulfur containing amino acids contribute substantially to the maintenance and integrity of cellular systems by influencing cellular redox state and cellular capacity to detoxify toxic compounds, free radicals, and reactive oxygen species. Met and Cys contribute significantly to the cellular pool of organic sulfur and generally to sulfur homeostasis. Genetic defects in the enzymes regulating sulfur pools produce a variety of human pathologies. In addition, thiol imbalance has been associated with multiple disorders, including vascular disease, Alzheimer's, HIV, and cancer. Sulfur containing amino acids, their metabolisms and role in human diseases, and possible treatments to restore the thiol balance have been reviewed.[123]

The discovery of the selenoproteins and antitumorigenic properties of selenium compounds focused attention on studies of biologically active selenocysteine containing peptides. Selenium analogues of sulfur containing amino acids have acquired increasing importance in the study of selenium poisoning and various detoxification studies. Efficient synthesis of cystine, selenocystine, and their higher homologues like homo and bishomo amino acid derivatives from natural amino acids using tetrathiomolybdate and tetraselenotungstate reagents under mild and neutral conditions has been reported.[124] The easy conversion of tosylates into disulfides and diselenides (Scheme 27) would be very useful in peptide synthesis where one can use Ser as a substitute for Cys avoiding the use of an additional protecting group and aerial oxidation of the sulfhydryl group.



Scheme 27

Protection of thiol groups is sometimes troublesome. Conjugate, hetero-Michael addition of thiols to triple bonds activated by electron-withdrawing groups to afford the one-to-one adduct is a well-known possibility, but it has limited scope. This is due to the harsh conditions reported for the protection or deprotection steps, unknown chemoselectivity, formation of *Z*/*E* mixtures, and/or double addition to give dithioacetals. The conjugate addition of aliphatic and aromatic thiols to ethynyl *p*-tolyl sulphone (tosylacetylene) has been managed to afford Tos-vinyl derivatives chemoselectively (Scheme 28) and stereoselectively (isomers *Z*) in practically quantitative yields.[125]



(i) $HC{\equiv}CTs$, Et_3N (1 mol%), CH_3CN/CF_3CH_2OH, O °C, 30 min

Scheme 28

Protection of Cys in Fmoc solid phase peptide synthesis[126] and chemoenzymatic synthesis of optically active phosphinic analogues of *S*-substituted sulfur-containing amino acids (homoCys and Met)[127] has been discussed. The organophosphorus analogues of the biologically significant sulfonium compounds *S*-adenosyl-Met and *S*-methyl-Met are much more stable than their carboxylic prototypes.[128] Cystalysin from the oral pathogen *Treponema denticola* is a pyridoxal 5′-phosphate (PLP)-containing enzyme which catalyzes the β-displacement of the β-substituent from both L-Asp and L-Cys sulfinic acid. Possible mechanism and physiological implications have been discussed.[129]

Selenium (Se) is an essential element for animals and possibly for plants, but its physiological nature in humans is ambivalent since it can cause disease by deficiency, but is toxic at levels relatively close to those required for health. The complexity of selenium (Se) chemistry in the environment and in living organisms presents broad analytical challenges. The selective qualitative and quantitative determination of particular species of this element is vital in order to understand selenium's metabolism and significance in biology, toxicology, clinical chemistry, and nutrition. This calls for state-of-the-art analytical techniques such as hyphenated methods that are reviewed with particular emphasis on interfaced separation with element-selective detection and identification of the detected selenium compounds. Gas chromatography with atomic emission detection of ethylated species and fluoroacid ion pair HPLC have revealed the presence of a previously unrecognised Se—S amino acid, *S*-(methylseleno)Cys. Selective detection and identification of Se containing compounds and recent developments have been reviewed.[130]

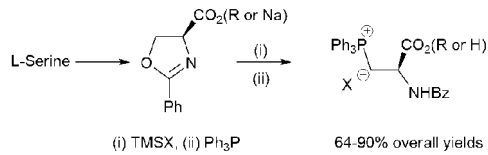
Tryptophan synthase is a bienzyme complex that catalyzes the final two steps in the biosynthesis of L-Trp in bacteria and plants. It has been used in the synthesis of a wide variety of Trp analogs, as well as other unnatural L-amino acids. The β-subunit catalyzes the condensation of indole with L-Ser to give L-Trp. The β-reaction has been used for the preparation of a wide range of analogues of L-Trp, as well as aza, thia, and selena heterocyclic analogues. In addition, S-alkyl and S-aryl cysteines, Se-alkyl selenocysteines and β-nitrogen substituted alanines have been prepared.[131] The use of disposable gold working electrodes for cation chromatography–integrated pulsed amperometric detection of sulfur-containing amino acids is discussed[132] in Section 6.2.

3.12 Phosphorus-containing α-amino acids

The key role of α-amino acids in the chemistry of life and as structural units in peptides and proteins has led to intense interest in the chemistry and biology of their mimics. An important class of such mimics are the α-aminophosphonic acids, which are analogues of α-amino acids in which the carboxylic group is replaced by a phosphonic function. These compounds are extremely important antimetabolites. However, the replacement of the carboxylic functionality with the phosphonic one has a number of important consequences as regards to the structure and the acid/base properties of this family of α-amino acid mimics. The same considerations apply to another family of P-containing molecules, such as phosphorylated α-amino

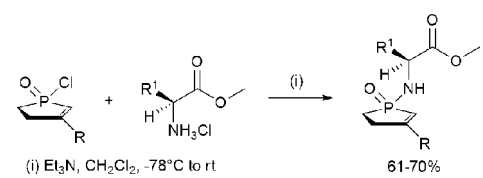
acids. The phosphorylation of certain amino acid residues, controlled by protein phosphatases and kinases, regulates a number of processes in living organisms, including metabolic pathways, membrane transport, gene transcription, and motor mechanisms. Ser, Thr, and Tyr and a number of other amino acid residues can be modified by the attachment of a phosphate group. The physiological impact of these modifications can be better elucidated after a detailed characterization and stereochemical identification of these P-containing molecules. Simple phosphonic acids are nonhydrolyzable analogues of phosphate esters in which the P–O ester bond is replaced with a nonhydrolyzable P–C bond ($RO–PO_3H_2$ *vs.* $R–PO_3H_2$). Phosphonic acids exist at physiological pH as their salts, called phosphonates and might be used as potential medicines in several diseases, including cancer and HIV. The therapeutic potential of phosphatase inhibition creates an interest in the design of potent and selective inhibitors, in mechanistic studies and structural investigations.

Isolation of 2-amino ethyl phosphonic acid from several organisms and human beings has clearly shown that amino phosphonic acids are biologically an important class of compounds. In order to achieve the synthesis of phosphonium salts, which are useful for the hemisynthesis of unusual amino acids by C=C bond formation, the stereospecific synthesis of β-halogeno amino acid derivatives bearing ester or acid functions by ring opening of the oxazoline derived from L-serine with trimethylsilyl halides has been described (Scheme 29).[133] The β-halogeno (-iodo or -bromo) derivatives were easily quaternized with triphenylphosphine to give the corresponding β-phosphonium salts in excellent overall yields from L-Ser hydrochloride.



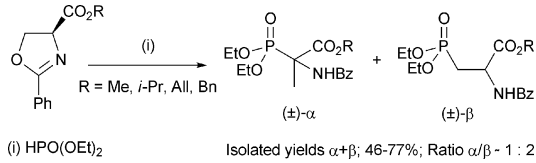
Scheme 29

Acid derivatives of phospholene oxides are a new class of compounds and expected to be possessing of potential biological activities. A convenient synthetic approach has been established to prepare a new class of 1-L-α-amino acid derivatives of phospholene oxides by amination of (±)-1-chloro-2-phospholene-1-oxides with several optically pure L-α-amino acid esters (Scheme 30). The two diastereomers were successfully separated by column chromatography.[134]



Scheme 30

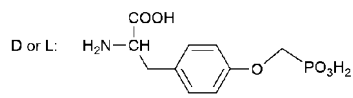
β-Phosphono Ala (AP3) and its derivatives are the P-analogues of Asp and are biologically important compounds, due to their antagonist activity on the central nervous system and their use in the synthesis of numerous enzyme inhibitors or modified peptides involved in viral maturation, cell development or infectivity. A new reaction of oxazolines derived from Ser with diethyl phosphite leading to ring opening products with P–C bond formation has been reported (Scheme 31).[135] This



Scheme 31

reaction, which proceeds under neutral conditions and without the use of any halogenated precursor, results in a mixture of racemic α- and β-phosphono alanines in an approximate 1:2 ratio. Since no significant transesterification occurs during the reaction, the amino acid derivatives obtained bear different protecting groups on the phosphonic and carboxylic acid functions, which is of particular interest for synthesis.

The unusual binding modes of phosphonates suggest that the presence of the ester (P–OR) oxygen atom in the substrate is important for proper binding in the catalytic complex. Incorporation of the methano-phosphonic acid moiety into D- and L-Tyr resulted in novel nonhydrolyzable phospho-Tyr analogues (**15**) and a series of related aryloxy (or thio) methyl and aryloxy (or thio) ethyl phosphonic acids of the general formula $RX–(CH_2)_n–PO_3H_2$ (where X = O or S and n = 1 or 2).[136] The advantage of aryloxymethyl and aryloxyethyl phosphonic acids over simple phosphonic acids is the reduction in pK_a values of the phosphonyl moiety.

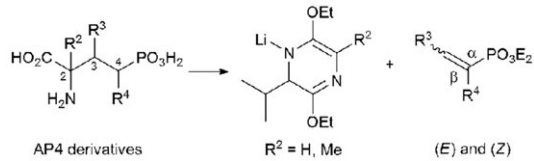


(**15**)

Phosphines and phosphinites are among the most important phosphorous-based ligands in organometallic chemistry with a wide range of steric and electronic properties. These ligands have found wide-spread applications in transition metal catalyzed asymmetric transformations. The metal–phosphorous bond are often stronger for phosphinites compared to the related phosphines due to the presence of the electron-withdrawing P—OR group. In addition, the empty σ*-orbital of the phosphinite $P(OR)R_2$ is stabilized and thus a better acceptor. The preparation of a series of the naturally occurring L-hydroxy-amino acid derived diphenylphosphinites (Boc-Ser($OPPh_2$)-OMe, Boc-Thr($OPPh_2$)-OMe, Boc-Tyr($OPPh_2$)-OMe, *Z*-Ser($OPPh_2$)-OMe, and *Z*-β-Ala-Tyr($OPPh_2$)-OMe) has been reported from commercially available amino acid precursors. These ligands readily bind to Pd(II) and Pt(II) forming the corresponding metal complexes.[137] The interaction of racemic 1-amino-3-(methylthio)propylphosphinic acid with benzylthiol catalysed by pyridoxal-5′-phosphate-dependent L-methionine-γ-lyase affords (*R*)-1-amino-3-(benzylthio)propylphosphinic acid, which was converted into the (*R*)-isomers of phosphinic analogues of homoCys and Met.[127]

As the principal excitatory neurotransmitter in the mammalian central nervous system, (*S*)-Glu plays a pivotal role in the regulation and maintenance of fast synaptic transmission and long-term potentiation/depression. Besides its physiological functions, Glu is involved in a variety of neuropathologies including epilepsy, stroke, nociception, cognitive disorders, and Alzheimer's disease. More recently, the G-protein-coupled receptors for glutamate have been discovered and perceived as valuable therapeutic targets. There is considerable interest in the development of asymmetric methodologies for the preparation of L-2-amino-4-phosphonobutanoic acid (L-AP4) derivatives that may result in useful tools for delineating the requirements for receptor binding. Conjugate additions of lithiated bislactim ethers derived from cyclo-[Gly-Val] and cyclo-[Ala-Val] to α-, β-, or α,β-substituted

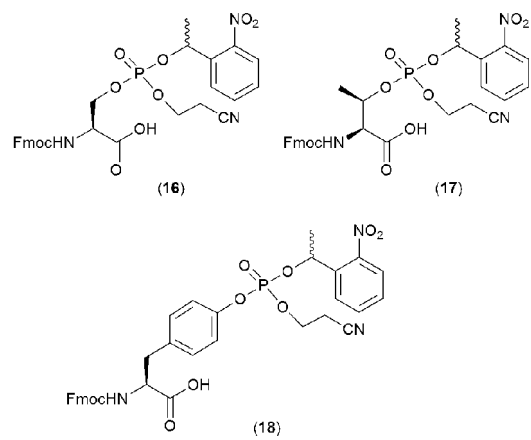
vinylphosphonates allowed direct and stereoselective access to a variety of 3- or 4-monosubstituted and 2,3-, 2,4-, or 3,4-disubstituted AP4 derivatives in enantiomerically pure form (Scheme 32).[138]



Scheme 32

The asymmetric Rh-catalyzed hydrogenation of enol esters with formation of chiral esters constitutes an alternative route to the enantioselective reduction of the corresponding prochiral ketones. Alkyl-substituted vinylcarboxylates, which normally show poor enantioselectivity in Rh-catalyzed hydrogenation with traditional chiral diphosphines, underwent highly enantioselective reactions with BINOL- and carbohydrate-based monophosphite ligands.[139] Since the vinyl carboxylates are accessible not only from ketones, but also by the Ru-catalyzed addition of carboxylic acids to alkynes, interesting synthetic possibilities are raised.

Kinase-mediated phosphorylation of Ser, Thr, and Tyr in peptides and proteins represents a central mechanism of cell regulation and is an area of intense study for which there is a need for new chemical tools. Caged compounds allow for spatial and temporal control over the release of a predetermined concentration of an effector molecule. A facile and efficient synthesis of 1-(2-nitrophenyl)ethyl-caged phosphoamino acids has been presented. The most common naturally occurring phosphoamino acids (Ser, Thr, and Tyr) were prepared as protected caged building blocks (**16**, **17**, and **18**, respectively) by modification with a unique phosphitylating reagent. The availability of such building blocks allows the synthesis of any peptide sequence using standard Fmoc-based SPPS techniques.[140]



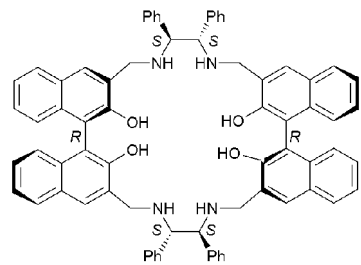
L-Phosphonomethylphenylalanine (L-Pmp) is an important phosphatase-resistant pTyr analogue. A most concise and stereoselective approach to the synthesis of the suitably protected Fmoc-Pmp(Bu^t)$_2$-OH was developed in order to incorporate the functionally significant L-Pmp residue into peptides and peptidomimetics. A series of potent Pmp-containing Grb2-SH2 domain antagonists have been synthesized, which can be used as chemotherapeutic leads for the treatment of certain breast cancer.[141]

Aberrations in protein-tyrosine-kinase (PTK)-dependent signaling are associated with proliferative disorders, including certain cancers. Accordingly, recent studies

showing the promise of kinase inhibitors have begun to validate PTK signaling blockade as an efficacious anticancer therapeutic approach. Src homology 2 (SH2) domains provide connectivity in PTK-dependent signaling through their high affinity association with phosphotyrosyl (pTyr)-containing peptide sequences. A novel and direct synthesis of (±)-(*rel*-1*R*,2*R*,5*S*)-3-acetyl-1,2,3,4,5,6-hexahydro-8-*O*-phosphoryl-1,5-methano-3-benzazocine-2-carboxylic acid methyl ester as a monomeric pTyr-mimicking analogue that constrains three torsion angles has been reported.[142] An important aspect of the current synthetic approach is its ability to prepare this new constrained pTyr mimetic in the absence of a potentially undesirable methyl substituent at the bridgehead 1-position.

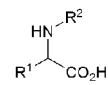
3.13 Labelled amino acids

Development of molecule-based enantioselective fluorescent sensors is receiving growing research attention because such sensors can potentially provide a real time technique to determine the enantiomeric composition of chiral molecules. Application of this method will be of great significance in the combinatorial high throughput assay of chiral drugs and catalysts. Bisbinaphthyl-based macrocycles are found to carry out enantioselective fluorescent recognition of α-amino acid derivatives (**19**). It is observed that one enantiomer of a *N*-protected phenyl-Gly can increase the fluorescence intensity of the binaphthyl fluorophores, but the other enantiomer does not cause much fluorescence enhancement. This highly enantioselective fluorescent response makes the binaphthyl macrocycles practically useful for the enantioselective fluorescent recognition of the amino acid substrate.[143]



(**19**)

Enantioselective fluorescent recognition of amino acid derivatives

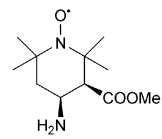


α-Amino acids labeled with short-lived positron emitters carbon-11 ($t_{1/2}$ = 20 min) and fluorine-18 ($t_{1/2}$ = 110 min) have been extensively used for the visualization of tumors, measurements of protein synthesis rates, and amino acid decarboxylation rates in humans using positron emission tomography (PET). No-carrier-added [1-^{11}C]α-amino acids D- and L-[^{11}C]Phe and [^{11}C]Tyr were synthesized from [^{11}C]CN^- using a simple one-pot two-stage procedure, a modified Bucherer-Strecker synthesis. The purification and chiral separation were achieved by the combination of solid-phase extraction and chiral HPLC to afford individual enantiomers of each amino acid. Because of its simplicity and wide applicability, the described procedure could be the method of choice to produce [^{11}C]amino acids for PET studies.[144]

Radiolabeled amino acids and PET show great potential for more accurate diagnosis of cerebral gliomas. A number of attempts were successful to label amino acids with ^{18}F, but only some of these unusual amino acids exhibit protein incorporation. The amino acid derivative O-(2-[^{18}F]fluoroethyl)-L-Tyr (FET) was not incorporated into protein, but can be produced with high radiochemical yields

due to nucleophilic substitution and thus offers practical advantages for routine clinical practice.[145] A fully automated preparation of S-(2-[^{18}F]fluoroethyl)-L-Met[146] and O-(3-[^{18}F]fluoropropyl)-L-Tyr (FPT),[147] amino acid tracers for tumor imaging with positron emission tomography, has been described. O-(3-[^{18}F]fluoropropyl)-L-Tyr (FPT), an analogue of O-(2-[^{18}F]fluoroethyl)-L-Tyr (FET) as an amino acid tracer for tumor imaging with PET was synthesized and evaluated. FPT was prepared by [^{18}F]fluoropropylation of L-Tyr in a two-step procedure.[148] A new ^{18}F-labeled branched amino acid, 2-amino-4-[^{18}F]fluoro-2-methylbutanoic acid (FAMB), has been prepared by using no-carrier-added [^{18}F]fluoride.[149] Chiral separations of fluorescamine-labeled amino acids have been characterized and optimized on a microfabricated capillary electrophoresis device. The results demonstrate the feasibility of combining fluorescamine labeling of amino acids with microfabricated CE devices to develop low-volume, high-sensitivity apparatus and methods.[150]

Amination of 3-carboxymethyl-1-oxyl-2,2,6,6-tetramethyl-4-piperidone with (*R*)-α-methylbenzylamine, $NaBH_3CN$ reduction of the resulting enamine, and removal of the chiral auxiliary from the separated diastereoisomers led to enantiomerically pure (3*S*, 4*S*) and (3*R*, 4*R*) methyl 4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylic acid (TOAC).[151] Enantiopure (3*S*,4*S*) H-β-TOAC-OMe (**20**) and (3*R*, 4*R*) H-β-TOAC-OMe, as well as their *N*-Fmoc derivatives have been synthesized.



(3S, 4S) H-β-TOAC-OMe

(**20**)

Proteomic studies demand new scalable and automatable MS-based methods with higher specificity and accuracy. An accurate and efficient method has been described for both precise quantification and comprehensive *de novo* identification of peptide sequences in complex mixtures. The unique feature of this method is based on the incorporation of deuterium-labeled (heavy) lysines into proteins through *in vivo* cell culturing, which introduces specific mass tags at the carboxyl termini of proteolytic peptides when cleaved by certain proteases. The *in vivo* lys-d_4 tagging is fully amenable to automation for a large-scale analysis and is particularly useful for the systematic investigation of complex proteomes such as the human proteome.[152]

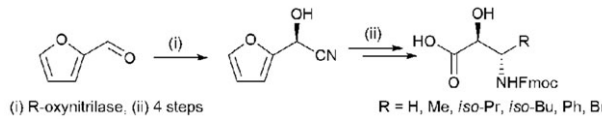
Synthesis and evaluation of substituted benzophenone photolabeling amino acids for the methionine proximity assay has been discussed.[153]

3.14 Synthesis of ß-amino acids and higher homologous amino acids

Although less abundant than α-amino acids, β-amino acids are also present in nature. While only a few occur in mammals, an increasing number is found in bacteria, sponges, and plants. In the latter cases, the β-amino acids are not only catabolites, but also constituents of a variety of compounds that exhibit interesting biological properties. During the last years β-amino acids have gained considerable attention due to their antibiotic, antifungal, cytotoxic, and other important pharmacological properties. β-Amino acids are key components of many naturally occurring peptides, too. The replacement of α-amino acids in biologically active peptides by certain β-counterparts can have pronounced effects on their folding properties, resulting in modified biological properties of the unnatural analogues. Currently, the synthesis of oligopeptide chains of β-amino acids is attracting much interest because of their ability to fold into defined three-dimensional structures. As

a result, much effort has been made to develop efficient methods for the preparation of this class of compounds.

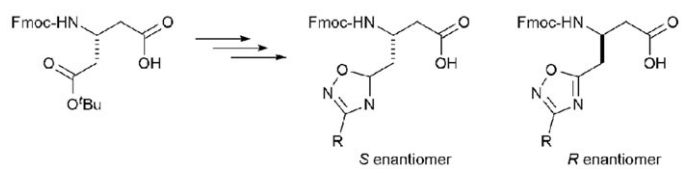
To obtain the Fmoc-protected (2*S*,3*S*)-2-hydroxy-3-amino acids, an earlier described chemoenzymatic route was further developed.[154] After the formation of cyanohydrin from 2-furaldehyde in the presence of R-oxynitrilase and subsequent protection, it was transformed into fully protected ethanolamines. Ozonolysis provided the target compounds in good yields (Scheme 33). The α-hydroxy-β-analogues of Gly, Ala, Val, Leu, phenyl-Gly, and Phe have been prepared by using this method.[154]



Scheme 33

Syn-α,β-Dialkyl β-amino acids are interesting building blocks because of their sheet-forming propensity and presence in bioactive compounds. Their derivatives suitably protected for solid-phase synthesis have been reported and give rise to residues containing positively charged Lys-like side chains. These amino acids, as well as *syn*-α,β-dialkyl β-amino acids that contain diverse hydrophobic side chains, were prepared.[155]

β-*N*-tert-butyloxycarbonyl-*N*-carboxyanhydrides are very reactive β-amino acid derivatives and reacted with different nucleophiles like aminoesters, enolates, *N*-methyl-D-glucamine, amidoximes to afford in good to excellent yields peptides, β-amino ketocompounds, β-aminosugars and functionalized disubstituted 1,2,4-oxadiazoles.[156] A new series of ß^3-amino acids were efficiently prepared by using **Arndt–Eistert** homologation of α-amino acid derivatives. This method proceeds stereospecifically to a high degree in most cases and suitable conditions have been found for a variety of N^{α}-protecting groups, including Fmoc.[157] The symmetry of the resulting ß-HomoAsp (β-HAsp) moiety has been exploited to prepare both enantiomers, avoiding the use of the expensive D-Asp derivative (Scheme 34). This strategy might be used for the synthesis of various β-HAsp-derived β^3-amino acids.



Scheme 34

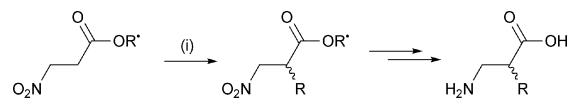
An asymmetric synthesis of a protected derivative of a new helix-forming β-amino acid, *trans*-4-aminopiperidine-3-carboxylic acid has been reported. All intermediates were purified by crystallization. An analogous route provides either enantiomer of *trans*-2-aminocyclohexanecarboxylic acid.[158]

A bisphosphepine ligand with stereogenic phosphorus centers for the practical synthesis of β-aryl-β-amino acids by asymmetric hydrogenation has been reported.[159] A catalytic asymmetric procedure for the preparation of β-amino acids (specifically β-substituted Asp derivatives) is reported. The *Cinchona* alkaloid catalyst benzoylquinine (BQ) mediates up to five distinct steps of a reaction pathway,

all in one reaction vessel. The products of this reaction, highly optically enriched β-substituted Asp derivatives, were prepared from *N*-acyl-α-chloro-Gly esters and acid chlorides in the presence of the catalyst.[160]

Five- and six-membered cyclic β-amino acids have been prepared in a short and stereoselective manner. The synthesis features a diastereoselective thioester enolate/imine condensation reaction and a ring-closing metathesis as key processes.[161] Four novel β-amino acids bearing the canonical nucleobases guanine, cytosine, adenine, and thymine in the side chain, are synthesized starting from BOC-L-Asp-4-benzyl ester. The syntheses are accomplished in six steps by the nucleophilic substitution of (*S*)-β-(tert-butoxycarbonylamino)-δ-bromopentanoic acid benzyl ester with the corresponding nucleobase derivative as the key step.[162]

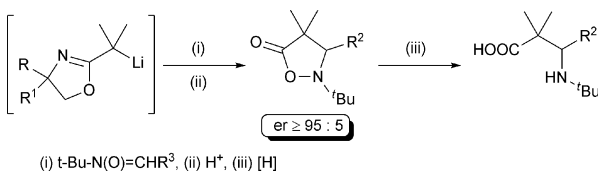
Enantiopure β-amino-γ-hydroxy acids and γ-amino-δ-hydroxy acids have been prepared by stereodivergent synthesis from L-Asp and L-Glu, respectively. The stereochemistry at the carbon atom attached to the amino group was determined from the starting material, but the configuration at C-4 or C-5 is controlled by diastereoselective alkylation with diethylzinc or ethylmagnesium bromide.[163] While quite a number of synthetic approaches towards enantiopure α- and β^3-amino acids are known, facile synthesis routes to the 2-branched β-amino acids are rare and limited to single compounds. Phosphoramidite-derived copper(I) catalysts readily facilitate the enantioselective 1,4-addition of dialkyl zinc reagents to nitro acrylates. The resulting 2-substituted 3-nitro propionic acid esters can easily be transformed to β^2-amino acids (Scheme 35).[164] This process is a promising approach towards enantiomerically pure 2-branched β-amino acids.



Scheme 35

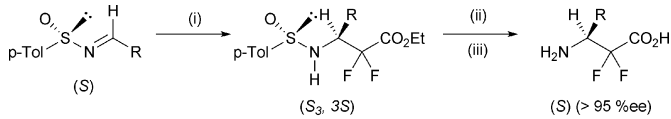
Catalytic enantioselective synthesis of β-amino acid derivatives was presented as the first enantioselective catalytic method for the synthesis of γ-unsaturated β-amino acids.[111] A novel and practical chiral catalytic method has been developed for the synthesis of α,β —disubstituted-β-amino acids in good overall yields and enantioselectivity. The availability of highly enantioenriched isoxazolidinones provides access to *syn*-disubstituted as well as α,α,β-trisubstituted compounds by base-mediated inversion or alkylation protocols.[165]

Isoxazolidinones are well-established building blocks in synthetic organic chemistry. A new simple and stereoselective synthesis of 5-isoxazolidinones based on the reaction of lithiated 2-isopropyl-2-oxazolines with nitrones was described. A chiral version of such a methodology allows the preparation of highly enantioenriched 5-isoxazolidinones which are useful precursors for the synthesis of β-amino acids (Scheme 36).[166]



Scheme 36

The enantiopure Davis' *N*-sulfinylimines were found to be efficient as chiral imine equivalents in the high-temperature Reformatsky-type additions with



R = C_6H_5 (**a**), 4-MeO-C_6H_4 (b), 4-F-C_6H_4 (**c**), 4-Cl-C_6H_4 (**d**), 4-CF_3-C_6H_4 (**e**), 2-furyl (**f**), *n*-C_5H (**g**), i-Pr (**h**), *t*-Bu (**i**)

(i) BrF_2CO_2Et, Zn, THF, reflux; (ii) 6 M HCl; (iii) methyl epoxide

Scheme 37

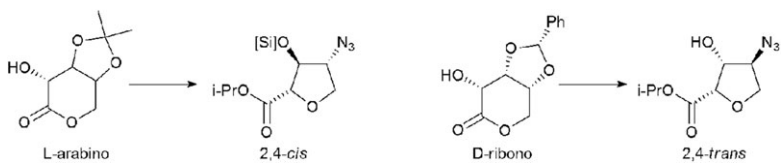
$BrZnCF_2COOEt$ affording an efficient approach to the enantiomerically pure α,α-difluoro-β-amino acids (Scheme 37). High chemical and stereochemical yields render this method useful for preparing the target amino acids.[91]

The stereoselective synthesis of novel α-epoxy-β-amino acids is described by a route that combines the chemistry of oxazolinyloxiranyllithiums with that of nitrones. The intermediate trioxadiazadispiro[2.0.4.3]undecanes have been isolated and converted by hydrolysis into epoxy-5-isoxazolidinones which can be transformed into the α-epoxy-β-amino acids by N–O reduction.[167]

A useful protocol has been developed for preparing novel *N*-protected β-amino nitriles as substrates for two nitrile-converting microorganisms.[168] 1-Benzoyl-2(*S*)-*tert*-butyl-3-methylperhydropyrimidin-4-one, a useful starting material for the enantioselective synthesis of α-substituted β-amino acids has been prepared in enantiomerically pure form from (*S*)-Asn.[169] A variety of functionalized β- and γ-amino acids were synthesized as Gly transport inhibitors and their structure—activity profiles have been described.[69] The synthesis of enantiopure γ-substituted γ-amino acids with proteinogenic side chains, starting from the corresponding natural α-amino acids, was studied. *N*-Protected amino aldehydes containing various protective groups were directly reacted with methyl, benzyl, and tert-butyl phosphoranylidene acetate to produce α,β-unsaturated γ-amino esters. Simultaneous hydrogenation of the double bond and removal of either the benzyl or benzyloxycarbonyl group led to *N*- or *C*-protected γ-amino acids in high yield.[170]

Recently, β-hydroxy-γ-amino acids have received considerable attention, notably those acting as key components of peptidomimetic protease inhibitors. Asymmetric syntheses of the *syn*- and *anti*-stereoisomers of *N*-Boc statine, based on the stereodivergent reduction of a single sulfoxide derived from *N*-Boc-L-Leu, have been reported.[171]

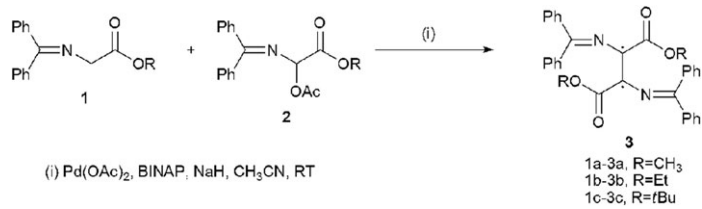
Short syntheses of enantiomeric templated scaffolds of *cis*- and *trans*-tetrahydrofuran γ-amino acids from pentono-δ-lactones derived from arabinose and ribose were reported (Scheme 38).[172]



Scheme 38

Routes to protected forms of all diastereomeric methyl 4-amino and 6-amino-2,5-anhydro-3-deoxy-hexonates have been described starting from either *gulono*- or *mannono*-1,4-lactones. Since both enantiomers of these starting materials are readily available, this work demonstrates the formal synthesis of *all* eight stereoisomers of both the 4-amino- and 6-amino-THF-2-carboxylate scaffolds (THF-templated γ- and δ-amino acids).[173]

α,β-Diamino acids are components of several peptidic antibiotics and other biologically interesting targets. 3-Aminoaspartic acid derivatives have been synthesized in high chemical yields and moderate stereoselectivities through a palladium-catalyzed π-azaallylic substitution reaction (Scheme 39).[174]



Scheme 39

Oxetin is a naturally occurring antibiotic with the unique structure of a *cis*-β-amino acid containing an oxetane ring. The preparation of a series of 2,3-*cis* and -*trans* β-azidoesters as new peptidomimetics and as analogues of the natural antibiotic oxetin has been described. These syntheses depend on the consistent high yield of S_N2 nucleophilic displacements of triflates by oxygen and nitrogen nucleophiles in the oxetane ring. It is noteworthy that there are no competing elimination reactions.[175]

Diastereoselective conjugate addition of lithium (*S*)-*N*-allyl-*N*-α-methylbenzylamide to a range of α,β-unsaturated esters followed by ring closing metathesis was used to afford efficiently a range of substituted cyclic β-amino esters. After deprotection, application of this methodology affords (*S*)-homoPro, (*S*)-homopipecolic acid, (*S*)-coniine, and (1*S*,2*S*)-*trans*-pentacin.[176]

3.15 Resolution of amino acids (Enantioseparation)

Separation of chiral intermediates is of tremendous importance in a number of fields. As new single stereoisomer drugs are developed in the pharmaceutical industry, the need for new chiral separation methods increases. Generally, there are two ways for the production of single enantiomers: one is asymmetric synthesis using chirally active catalyst, and the other is kinetic resolution of racemic mixtures using various separation methods. These conventional methods have been known to have advantage and disadvantage at the same time, *e.g.* HPLC is excellent for optical resolution, but the sample amount that can be separated at one time is usually very small. So, it is not economically efficient, considering its operating costs. Since technically recrystallization can also be applied, but some racemic mixtures cannot be separated by it, the application of *N*-acyl-D-amino acid amidohydrolase is one of the most useful and convenient methods for the production of D-amino acids at present. Recent progress in the study of structure—function relationships from the standpoint of improving this enzyme for industrial application have been summarized and discussed.[177]

One of the main difficulties of chiral separation of pharmaceuticals by membrane ultrafiltration is the low separation factor compared to conventional chromatography or affinity chromatography. The use of immobilized DNA membranes for chiral separation of Phe has been investigated.[178] Optical resolution by enantioselective membranes is relatively new and promising process for producing single enantiomers from racemic mixtures. It is very efficient for the separation of a certain racemic mixture but it has a pretty narrow application range. The enzymes used for the membrane reactors also have to be exchanged into new one regularly since they have a certain lifetime. For the optical resolution of α-amino acids (Trp and Tyr), enantioselective membranes crosslinks were prepared using sodium alginate (SA)

and chitosan (CS) as membrane materials.[179] Resolution of a racemic mixture of Phe and Met has been studied by using a supported liquid membrane.[180] The chiral carrier and transition metal were *N*-decyl-(L)-hydroxy-Pro and copper(II).

A methodology for the enzymatic resolution of sterically constrained C^{α}-fluoroalkyl substituted amino acids has been developed. Racemic *H*-($^{\alpha}$Tfm)Ala-NH_2, *H*-($^{\alpha}CF_2Cl$)Ala-NH_2 and *H*-($^{\alpha}CF_2Br$)Ala-NH_2 were separated with very high enantioselectivity using amidase from *Mycobacterium neoaurum*, yielding the corresponding (*R*)-acids. Furthermore, the first example of an enzymatic resolution of a C^{α}-fluoroalkyl substituted Phe derivative has been established using amidase from *Ochrobactrum anthropi*.[100] The enzyme BSL2, a highly active lipase, is used in the kinetic resolution of *N*-(2-ethyl-6-methylphenyl)alanine from the corresponding racemic methyl ester. Reaction conditions are optimized to enhance the enantioselectivity.[181] A lipase-catalyzed method for the dynamic kinetic resolution of Pro and pipecolic acid methyl esters was developed based on the acylation of the secondary amino group of the amino esters with vinyl butanoate by *Candida antarctica* lipase A.[182]

Most inhibitors of angiotensin converting enzyme (ACE) contain an L-homoPhe ethyl ester moiety in their structure. Despite the convenience of chemo-enzymatic methods in synthetic organic chemistry, some uneasiness still exists among synthetic chemists regarding the handling of enzymes. Furthermore, most commercially available enzymes are expensive and can be unstable, unless kept under specific storage conditions. Most of these drawbacks could be avoided by using crude enzymatic preparations; however, the use of these raw preparations is much less explored than the use of pure enzymes. A simple protocol has been proposed for the preparation of both enantiomers of homoPhe by enzymatic resolution using kidney acetone powders of different mammalian species.[183] *N*-Acyl-esters of mixtures of D-alloisoleucine and L-Ile were easily hydrolysed by enzymatic catalysis (alcalase) in water to the *N*-acyl-L-forms allowing the recovery of the D-allo stereoisomer in high yield. In fact, both *N*- or *C*-protected forms of D-allo stereoisomer can easily be prepared in one step from the material surviving to enzymatic hydrolysis.[184]

Stable nitroxide free radicals are of continuing interest for use as spin labels in the study of conformation and structural mobility of biological systems, as spin traps of other radical species and as oxidizing agents. 4-Amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylic acid (β-TOAC), the first spin-labelled, cyclic, chiral β-amino acid has been resolved in an enantiomerically pure state, and the *N*-Fmoc derivatives of the enantiomers have also been synthesized.[151] However, the TOAC structure presents a few disadvantages: its achiral character may hinder access to stereochemical information, and its tetrasubstituted α-carbon induces a reduced reactivity of the amino function which may be problematic if the residue is to be placed at an internal position of a peptide. A spin-labelled cyclic β-amino acid, *trans* 3-(9-fluorenylmethyloxycarbonylamino)-1-oxyl-2,2,5,5-tetramethylpyrrolidine-4-carboxylic acid (Fmoc-POAC-OH) has been developed. The cyclic structure was expected to allow easier peptide couplings than the TOAC structure. Enantiopure forms could be obtained upon esterification with (*aR*)-1,1′-binaphthyl-2,2′-diol, chromatographic separation of the obtained diastereomers, and facile saponification of the aryl ester function with removal of the chiral auxiliary.[185] Direct and indirect high-performance liquid chromatographic (HPLC) methods were developed for the enantioseparation of spin-labelled, cyclic, chiral β-amino acids containing nitroxide free radicals and their *N*-Fmoc-protected analogues.[186]

Control of selectivity in the enantiomeric separation of three aromatic amino acids (Phe, Tyr, and Trp) was demonstrated utilising two separate electrolyte additives. Sulfated-p-cyclodextrin was chosen as the chiral selector while the addition of dextran sulfate provided a fine-tune separation selectivity. The two additives were found to interact independently with the amino acids.[187] Maltodextrins are complex mixtures of malto–oligo and polysaccharides obtained from starch by partial acid or enzymatic hydrolysis and have been used as chiral selectors in CE as well as for the

design of enantioselective, potentiometric membrane electrodes. These electrodes could be reliably utilized in the assay of L-Pro as raw material, with the best enantioselectivity and time-stability.[188]

An efficient resolution of *rac*-2-cyano-2-methyl-3-phenylpropanoic acid led to a large laboratory scale synthesis of (*S*)-α-methyl-Phe from benzaldehyde and methyl cyanoacetate.[83] Ligand-exchange micellar electrokinetic capillary chromatography was used for the chiral resolution of underivatized and dansyl amino acid enantiomers simultaneously.[189] Dynamic kinetic resolution was used for asymmetric synthesis of α-alkyl amino acids *via* dual-function catalysis of modified *Cinchona* alkaloids which catalyze both the racemization and alcoholytic kinetic resolution of alkyl *N*-carboxyanhydrides (NCA) bearing an electron-withdrawing *N*-protecting group.[190]

Immobilization of chloro-*s*-triazines bearing amines or amino acid derivatives as chiral selectors on solid supports such as aminopropylsilica provided a series of chiral stationary phases enabling the enantioresolution of amino acid derivatives. A series of chiral derivatizing reagents was synthesized in which one chlorine in cyanuric chloride is substituted by an alkoxy or aryloxy group, whereas the second chlorine is replaced by an L-amino acid derivative (amide or *tertiary* butyl ester). These chiral derivatizing reagents were assessed for their suitability in derivatizing DL-amino acids followed by liquid chromatographic separation of diastereomers.[191] A new ligand exchange chiral stationary phase (CSP) has been developed by covalently bonding (*R*)-*N*,*N*-carboxymethyl undecyl phenylglycinol mono-sodium salt onto silica gel and applied in the resolution of α- and β-amino acids.[192]

The enantiomeric resolution of certain 2-arylpropionic acids was achieved on thin silica gel plates impregnated with optically pure L-(–)-Ser as chiral selector. Resolution in the presence of water assumes the possibility of participation of some kind of hydrogen bonding, and electrostatic interactions between COO^- of the compounds and $-NH_3^+$ of Ser.[193]

To obtain optically active *threo*-2-amino-3-hydroxy-3-phenylpropanoic acid, (2*RS*, 3*SR*)-2-benzoylamino-3-hydroxy-3-phenylpropanoic acid was first optically resolved using (1*S*, 2*S*)- and (1*R*, 2*R*)-2-amino-1-(4-nitrophenyl)-1,3-propanediol as the resolving agents.[194]

To synthesize D-amino acids, many useful methods, such as chemical, fermentative, and enzymatic production, have already been developed. In case of chemical syntheses, D-amino acids are produced by the chiral resolution of DL-amino acids; however, these systems have a low yield and high cost. Fermentation methods have hardly been applied to produce D-amino acids. Enzymatic biotransformations, which produce optically pure D-amino acids from DL-racemic mixtures without any by-products from D-amino acid-specific enzymes, would appear to be the most feasible method for the production of D-amino acids with regard to high optical purity and productivity. The thermostable D-methionine amidase exhibited a high amidase activity and D-stereospecificity toward D-amino acid amides and esters, yet did not hydrolyze D-peptides.[195]

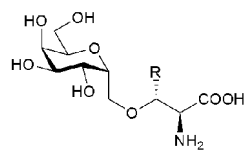
3.16 Sugar amino acids and derivatives

Glycosylation is an ubiquitous post-translational modification of proteins and is associated with a number of processes both within cells and at cell surfaces. Aberrant glycosylation of cellular proteins and glycolipids is associated with various diseases, including cancers and inflammatory conditions. Therefore, an understanding on a molecular level of the structural features of glycoproteins that are recognized by various enzymes and receptors would be valuable in developing inhibitor-based strategies to control carbohydrate-mediated cellular processes. This fundamental understanding could, in turn, lead to new therapeutic strategies for conditions that are characterized by abnormal glycosylation.

Sugar amino acids represent highly substituted polyfunctionalised building blocks. Carbohydrate moieties have influence on conformation, solubility and

stabilisation of proteins. To improve the metabolic stability of potential drugs, a lot of more stable C–C-linked analogues have been synthesised during the past two decades. In this respect, α,α-disubstituted sugar α-amino acids are subjects of exceptional interest because an additional substituent at the α-position sterically constrains the free rotation or conformation flexibility of their side chain or strictly fixes conformation by a saccharide ring. Hydrolysis of hydantoins provides a suitable route for the preparation of sugar α-amino acids. Although starting hydantoins can be obtained conveniently by the Bucherer–Bergs reaction, only a few papers on the application of this reaction to a carbohydrate derivative have been reported till now. The synthesis and structure determination of some Ser derivatives branched at C-2 atom with a saccharide moiety has been presented. The new compound was obtained from suitably 6-*O*-protected methyl α-D-*lyxo*-hexofuranosid-5-ulose.[196] The synthesis and structure determination of some glycoconjugate model compounds having C-2-linked α-amino acid attached to a carbohydrate backbone in the C-5 position has been presented.[197] Thus, 2-[methyl (4*R*)-β-L-erythrofuranosid-4-C-yl]-D-Leu) was obtained by acid hydrolysis of the isopropylidene group from a hydantoin derivative followed by basic hydrolysis of the hydantoin ring.

The lability of the glycosidic bond towards chemical and enzymatic degradation *in vivo* results in low bioavailability of carbohydrate derivatives and prevents their oral application. Due to their therapeutic potential, the synthesis of glycopeptides and their mimetics represents a dynamically developing field of research. Significant improvements in activity, bioavailability, stability, and solubility of peptides were achieved by their glycosylation. The synthesis of glyco mimetics, representing the corresponding natural structures and offering higher metabolic stability, has already received much attention, and further effort on their synthesis are expected to be undertaken in the future. A common approach is the application of *C*-glycosides, which are no longer susceptible to cleavage by glycosidases. Derivatives of Ser and Thr were prepared, which have a *C*-glycosidic linkage and a stable ether-bridge between the carbohydrate and the amino acid part, resulting in a linkage elongated by one methylene group. As potential lead structures for a new class of glycosidase inhibitors, the novel *O*-glycosyl amino acid mimetics 3′-*O*-[2,6-anhydro-D-*glycero*-L-gluco-heptitol-1-yl]-L-Ser and -L-Thr (**21**) were synthesized, employing regio- and stereoselective aziridine ring opening methodology. They proved to be stable in the presence of glycosidases.[198]



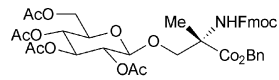
(21)

For the preparation of an *ortho*-carborane derivative bearing both carbohydrate and amino acid substituents, the key-steps of the synthesis are: opening of a glucofuranuro-gamma-lactone derivative with propargylamines, cycloaddition of decaborane to an acetylenic bond, and amidation with a *N*-Fmoc-glutamate derivative.[199] Cyclic oligomers composed of amide-linked furanoid and pyranoid epsilon-sugar amino acids were prepared by a cyclization/cleavage approach with the use of the oxime resin. These cyclic homooligomers were constructed by use of the known *N*-Boc protected furanoid epsilon-sugar amino acid and a novel pyranoid hydroxymethylene homologue. Conformational analysis showed that the side chains connecting the carbonyl functionality (*i.e.* C2) proved to be rigid, while the other side chains (C7) are conformationally flexible.[200]

C-Galactosyl and *C*-ribosyl β-amino acids were prepared by one-pot $InCl_3$-catalyzed Mannich-type three-component condensation (3CC) by combining the corresponding formyl *C*-glycoside, p-methoxybenzyl amine, and a ketene silyl

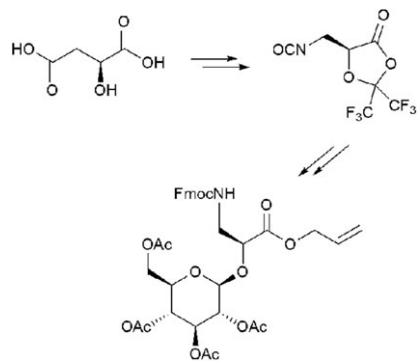
acetal. In each case the reaction was highly stereoselective and afforded only one single product in good to excellent yields.[201] A collection of 4-(*C*-galactosyl)- and 4-(*C*-ribosyl)-β-lactams featuring different substituents at C-3 and N-1 was prepared by combining in a one-pot procedure a formyl C-glycoside, a primary amine, and a substituted acetyl chloride in the presence of base (Staudinger-type reaction). Sulfonyl chloride and aminomethylated resins were used in sequence to remove excess of components and by-products. Two pure C-glycosyl-beta-lactams were effectively transformed into C-glycosyl-N-Boc-p-amino-α-hydroxy esters (C-glycosyl isoserines) and a C-ribosyl dipeptide *via* base-promoted heterocycle ring opening by methanol and L-Phe methyl ester, respectively.[202]

A number of glycoamino acids that contain conformational constraints have been synthesised and the majority of unnatural glycosylated amino acids reported are *C*-glycoside derivatives, including *C*-glycosylated α-amino acids that possess a quaternary center. The suitably protected glycosylated amino acid β-D-glucopyranosyl-(*S*)-α-MeSer (**22**) is an important building block in the synthesis of constrained glycopeptides in which the conformational restriction is only present in the amino acid moiety and can be used as an attractive building block for the synthesis of new, constrained glycopeptides.[203]



(**22**)

A new and convergent synthesis of a C-glycosylated phenylalanine derivative using palladium-catalyzed Stille and Neghishi cross-coupling reactions is described. The coupling product constitutes a precursor of a natural glycosylated tyrosine mime.[204] New types of glycoconjugates, *O*-glycosylated, *N*-glycosylated and *O*-,*N*-diglycosylated (*S*)-isoSer derivatives have been synthesized starting from hexafluoroacetone-protected malic acid. The new compounds represent glycosylated β-alanine surrogates which are suitable for β-peptide modification (Scheme 40).[205]

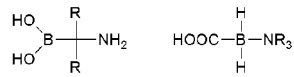


Scheme 40

3.17 Miscellaneous

In recent years there has been an increasing interest for new practical methods to prepare novel non-natural *R*-amino acid derivatives to serve as building blocks in combinatorial chemistry and drug discovery. Although many routes to amino acids have been developed, there is still a need for concise and convergent approaches that allow structure variability and facile incorporation of functional groups and ring

systems. As non-natural *R*-amino acid derivatives, boronic acids have assumed great importance since they serve as transition state analogues of natural amino acids. Diversity of reactions and the development of a new boron-based methodology for applications in organic synthesis of α-aminoboronic acids (**23**); α-aminoboronic derivatives of Gly, Pro, Ala, Orn, and Arg; amine-carboxyboranes (**24**); and their derivatives have been discussed and summarized.[206] A number of new substituted-borane adducts of amines and amino acids have been synthesized.[207]



(**23**) (**24**)

Photocatalytic reactions occurring at semiconductor particles/solution interfaces can be applied to organic syntheses. In a review article, examples of photocatalytic syntheses of cyclic amino acids by suspended semiconductor particles, *e.g.*, titanium(IV) oxide or cadmium(II) sulfide are introduced and interpreted.[208]

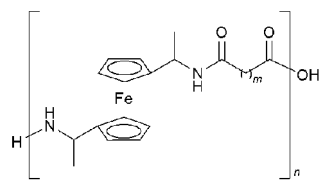
A synthetic strategy was developed to anchor L-Val to chloromethylated styrene-divinyl benzene co-polymer beads. The polymeric ligands containing bidentate N,O donor sites were treated with a solution of ruthenium(III)chloride,[209,210] palladium chloride,[211] manganese(II) acetate,[212] or Cu(II) acetate[213] to form the metal complex on the support. The complexes were used for catalytic oxidation of alkanes and alkenes. Polymer-supported Cu(II) complexes with L-Val and L-Phe were used as chiral catalysts for asymmetric epoxidation of nonfunctionalized straight-chain terminal olefins.[214] L-Phe was also anchored to P(S-DVB) resin and its complex with $PdCl_2$ was prepared. The newly synthesized catalyst was found to be a recyclable catalyst for the hydrogenation of 1-octene and acetophenone.[215]

The application of the polystyrylsulfonyl-3-nitro-1H-1,2,4-triazolide-resin (readily available from the corresponding commercially available polystyryl sulfonyl chloride resin), to the solution-phase synthesis of esters from protected α-amino acids in high yields and purity have been described, with a low level of racemisation of the amino acids. All by-products can be removed by filtration and extraction.[26] Four trityl-type amido-methyl polystyrene resins were evaluated comparatively in terms of their stability. These resins were also applied in parallel with the Wang resin in Fmoc solid phase synthesis. The best yield was obtained with the o-Cl-trityl-amidomethyl polystyrene resin.[216]

Synthesis of *N*- and *O*-acyl derivatives of DL-Ser and threo-DL-phenyl-Ser was accomplished by a regioselective acylation of the corresponding amino acid. The residues introduced into the amino acid structure contain hydrophobic long chain or aromatic moieties such as lauroyl, myristoyl and phenylacetyl.[217]

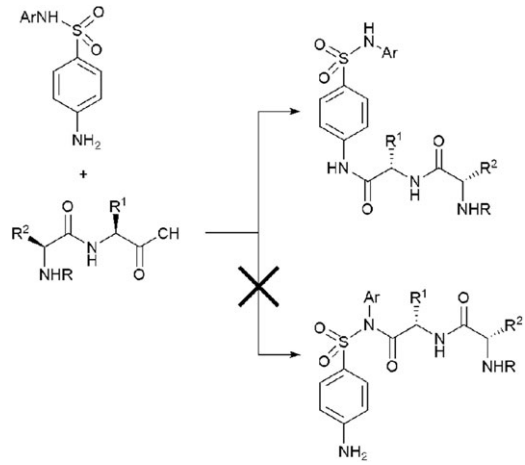
Acylation of antimalarial and bacteriostatic sulfonamides with *N*-protected amino acids was carried out using standard peptide coupling methods. These acylation reactions are regioselective for the N^4 nitrogen atom of diazine-containing sulfonamides (Scheme 41).[218]

Bioorganometallic chemistry is a recent propulsive discipline dealing with organo-metallic compounds coupled with biomolecules. The rational and convenient multistep syntheses of *N*- and *C*-protected ferrocene amino acids (**25**) have been described.[219]



m=0-3; *n*=1-6

(**25**)



Scheme 41

Some amino acids (DL-Phe, DL-Trp, His or Ala were reacted with nitroso compounds (*N,N*-dimethylamino-4-nitrosoaniline or *N,N*-diethylamino-4-nitroso-aniline) to form new amino acid azo compounds. These ligands interacted with Fe(II) ions in strong acidic media (pH 1) to form complexes. The amino acid azo compounds act as neutral and bidentate ligands by coordination through the α-nitrogen of the azo group and the carbonyl of the carboxylic group. The solubility of these complexes has been determined and discussed.[220] The unique chemistry of amino acid dithiocarbamates with Ru(III) bis-β-diketonates has been discussed. The direct reactions of the amino acids (Pro or NMeIle) with $[Ru(\beta\text{-diketonato})_2(MeCN)_2][CF_3SO_3]$ yielded $[Ru(dpac)_2(Pro)]_2$ and $[Ru(acac)_2(NMeIle)]_2$ and are the first examples of paramagnetic μ-amino acidato bridged Ru(III) dimers which could be converted to their corresponding chelated amino acidato monomeric complexes upon treatment with base. The formal potentials of all of the Ru(III) complexes reported were determined by cyclic voltammetry.[221] The influence of metal ion availability and utilization on RNA function and evolution is important in the design of experiments to generate new RNA activities. The RNA species SHR1 reacts with biocytin (ε-biotinoyl-L-Lys) in the presence of Ni^{2+} or Pt^{2+} to produce a metal-bridged complex that migrates more slowly than unreacted RNA in the presence of streptavidin on denaturing polyacrylamide gels.[222]

A liquid membrane as a bio-mimetic membrane has a high potential for application to the selective separation of bioactive materials. Calix[6]arene hexacarboxylic acid was found to be a useful carrier for transporting amino acids through a liquid membrane. The calix[6]arene, which has a cyclic structure to include a guest molecule of the amino acid ester and bears six ionizable carboxylic acids to contribute electrostatic interaction, exhibited a high transport efficiency compared to its monomer analog and the other calix[*n*]arene derivatives. The novel carrier successfully transported hydrophobic amino acid esters. It is concluded that the calix[6]arene is one of the best carriers currently available for the transport of amino acids.[223]

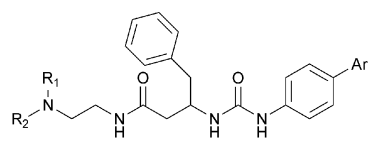
Adsorption from liquids into molecular sieve zeolites is finding increasing applications in separation and purification processes. Because of the hydrophobicity of zeolite, the pore volume of high silica zeolite will be free from water and is accessible for the adsorbate. Therefore, the emergence of hydrophobic zeolites such as silicalite and ZSM-5 finds application in the separation of carbohydrates and amino acids.[224] The anomalous 'salting-in' of neutral compounds of natural amino acids with transition metals has been discussed,[225] as an example of new biomimetic chemistry in water.

Cancer chemotherapy is ultimately limited by the toxicity of drugs to normal tissues. The majority of anticancer drugs have a detrimental effect on normal cells. Numerous attempts have been made to increase the effectiveness of anticancer drugs by increasing their concentration at the target site. Targeted delivery of drugs to cancer cells can theoretically allow the use of a reduced dose to achieve the same therapeutic response, with a consequent decrease in systemic toxicity. New biodegradable polymers are needed to improve the biodistribution and targeting-ability of polymeric carriers. The synthesis and characterization of branched poly(L-glutamic acid) (PG) containing multiple PG chains centered on a poly(amidoamine) (PAMAM) dendrimer or polyethyleneimine (PEI) cores were described.[226] Unlike conventional linear PG, these polymers had multiple arms of PG chains. These polymers are biodegradable and water-soluble, which make them attractive drug carriers for targeted delivery of chemotherapeutic agents, diagnostic agents, and gene products.

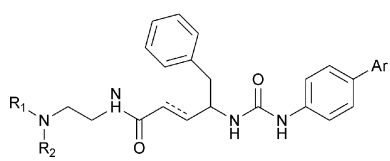
The feasibility of using palm oil fractions as cheap and abundant sources of raw material for the synthesis of amino acid surfactants was investigated and discussed. The lipase-catalyzed transacylation of palm oil fractions with amino acids is potentially useful in the production of mixed medium- to long-chain surfactants for specific applications.[227] Over the past several decades, the study of the hydrophobicity of biomolecules, particularly amino acids has resulted in the development of a variety of hydrophobicity scales. In a review, the various methods of measuring amino acid hydrophobicity and the literature on amino acid interactions have been discussed.[228]

The syntheses of non-electrolyte complexes of the general formula [Co(Hdmg)(2)(HA)X], where Hdmg = dimethylglyoximato monoanion, HA = glycine (Gly), Serine (Ser), Cysteine (Cys) or Cystine (Cys–Cys) and X = Cl, Br, I, SCN have been described.[229] Voltage-dependent calcium channels (VDCCs) mediate the influx of Ca^{2+} in response to membrane depolarization and regulate numerous intracellular functions including contraction, secretion, neurotransmitter release and gene expression. The synthesis and structure—activity relationship (SAR) study of L-Cys-based N-type calcium channel blockers have been described.[230]

The amino acid Gly is an important inhibitory neurotransmitter in the central nervous system (CNS). Several β- (**26**) and γ-amino acid (**27**) derivatives were prepared as glycine transport inhibitors and their ability to block the uptake of [^{14}C]-Gly in COS7 cells transfected with human glycine transporter-2 (*h*GlyT-2) were evaluated.[69]



β-amino acid derivatives
(**26**)



γ-amino acid derivatives
(**27**)

4. Physical and stereochemical studies of amino acids

4.1 Crystal structures

Studies on the crystal structures of amino acids have been of increasing significance to understand both the structures of proteins and the origin of life on earth. Techniques employed to determine amino acid structures have so far been limited mainly to X-ray diffraction methods and focused on the bulk representation, while investigation of the surfaces of amino acids is not only important for characterization of their crystal structures but also surface properties and crystallization processes. Atomic force microscopy (AFM) is unique in its ability to image the surface morphologies of a variety of organic and biological specimens at the nanometer scale directly. Since its invention, some surface lattice structures of amino acid crystals have been achieved successfully by AFM. However, many difficulties and problems such as the artifact features are usually encountered due to the instability of tip–sample interactions during measurement, and which must be identified and eliminated carefully. It is still a great challenge to obtain high-resolution images at the molecular level on surfaces of amino acids by AFM. Molecular structures at the surfaces of DL-Val and L-Ala single crystals have been observed by AFM.[231] The results demonstrate that AFM has the ability to probe the surface structures of biological crystals at a molecular resolution in both the lateral and longitudinal dimensions.

One of the problems central to the design of host compounds is the placement of substituents in positions that converge on the functional or binding sites of guest molecules. Understanding *in–out* conformational equilibria in macrocyclic compounds thus represents a challenging task. Macrocyclic bis(α-amino acids) *cis*- and *trans* were prepared from the selectively protected tris(hydroxymethyl)aminomethane. The X-ray structures of the free bis(amino acids) and/or of the corresponding Cu(II) complexes have been determined allowing an unambiguous configurational assessment. At the same time, conformational *in–out* dichotomy of the functional groups has been demonstrated in the bis(amino acids) as well as in their Cu(II) complexes.[232]

4.2 Nuclear magnetic resonance spectroscopy

A new tool for magnetic resonance, L-6-heptafluorobutyryl-5-hydroxy-Trp, was synthesized and investigated using an antibody to perfluoroalkyl moieties developed previously. Typical immunoassay methods were ineffective, so a new technique was developed which binds amines and amino acids to the walls of acid-functionalized cuvettes. The studies demonstrated that the compound followed the pathway of endogenous serotonin.[233]

Following optimization of experimental conditions, solid-state ^{14}N MAS NMR spectroscopy is shown to be a valuable tool for characterization and studies of amino acids. Experimental strategies for the acquisition of high-quality ^{14}N magic-angle spinning (MAS) NMR spectra of the simple amino acids, which exhibit ^{14}N quadrupole coupling constants (C_Q) on the order of 1.2 MHz, have been devised. These are the first useful ^{14}N MAS spectra reported for nitrogen compounds having a $C_Q(^{14}N)$ value in excess of 1 MHz.[234]

4.3 Spectroscopic studies

The investigation of photo-induced processes in amino acids, activated by vacuum ultraviolet (VUV) radiation, is relevant to several fields such as space chemistry. In particular, ionization processes are important as they play a dominant role in molecular radiation damage in the VUV photon energy range. Ionic fragmentation of the sublimated α-amino acids Gly, L-Ala, L-Pro, and L-Val has been studied using a time-of-flight mass spectrometer coupled to a He I lamp. Partial ion yields

(branching ratios) and kinetic energy releases for the fragments have been determined.[235]

A comparative study of the feasibility and efficiency of Raman spectroscopic detection of thin layer chromatography (TLC) spots of some weak Raman scatterers (essential amino acids such as Gly, L-Ala, L-Ser, L-Val, L-Pro, L-hydroxy-Pro, and L-Phe) was carried out using four different visible and near-infrared (NIR) laser radiations. Three types of commercial TLC plates were tested and the possibility of inducing surface enhanced Raman scattering (SERS) by means of Ag-sol was also investigated.[236]

The combination of FT-IR spectroscopy, X-ray diffractometry, BET surface area measurements, and thermal methods verified that the protonated forms of various amino acids can be intercalated into montmorillonite. The protonated forms of six amino acids (Gly, L-Ala, L-Trp, L-His, L-Met, and L-Lys) were ion-exchanged into Na-montmorillonite. A comparison of the FT-IR spectra of the host, the guests, and host–guest substances revealed that the guest ions were intercalated successfully. The spatial arrangement of the guest ions was modeled by semiempirical quantum chemical method.[237]

The interfacial behaviour of L-Phe at a Pt surface was studied using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), and electrochemical quartz crystal nanobalance (EQ CN) measurements,[238] while neodymium complexes with amino acids: $Nd(Ala)_3Cl_3 \cdot 3H_2O$, $Nd(Val)_3Cl_3 \cdot 3H_2O$, $Nd(Phe)Cl_3 \cdot 5H_2O$ and $Nd(Trp)_3Cl_3 \cdot 3H_2O$ have been synthesized and their photoacoustic (PA) spectra reported.[239]

4.4 Other physical studies

In recent years the number of data banks of thermodynamic properties of amino acid solutions that may be useful for the interpretation of the physically well-founded models of the processes *in vivo* has greatly increased. It is necessary for its elaboration to dispose of the experimental set of reliable data on activity coefficients of the high polar zwitterionic form of an amino acid. The activity coefficients of zwitterions of aliphatic amino acids (β-Ala and Val) and of the charged Glu have been determined by EMF methods with the aim of further approximation of their concentration dependence with the use of chemical models. Dependence of activity coefficients of amino acid zwitterionic form concentration of amino acid and ionic strength has been approximated by two-parametric Pitzer equation, and the contribution of parameters of interparticle interactions has been analysed. The experimental data covering the broad concentration interval can be used for the testing and elaboration of chemical models of amino acids solutions.[240]

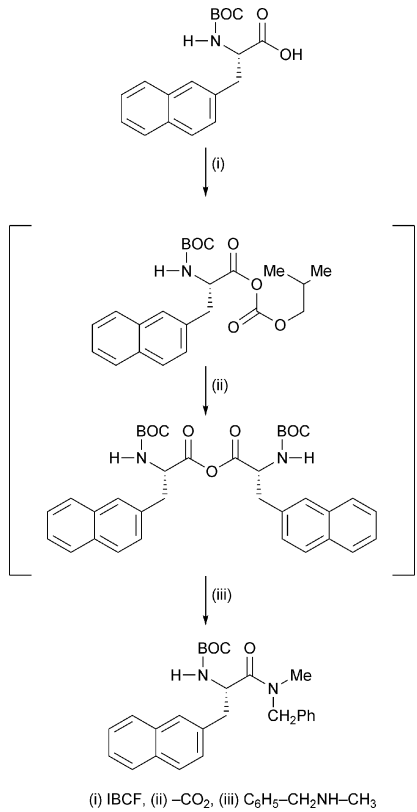
5 Chemical studies of amino acids

5.1 Racemization of amino acids

Amino acids with D-configuration were once considered as unnatural optical isomers, but several bacteria use D-amino acids as essential components of peptidoglycan in cell walls. However, advanced techniques for detection and separation of amino acid enantiomers make it easy to detect even small amounts of D-amino acids in biological samples. Recently, it has been discovered that a variety of free D-amino acids are widely distributed in various organisms, and evidence suggests that they play physiologically important roles. Free D-Ala was detected in a cell extract of the fruit-body of an edible basidiomycetous mushroom, *Lentinus edodes* (Shiitake) by means of RP-HPLC. The ability of the enzyme to catalyze the racemization of various D-amino acids was investigated. The enzyme catalyzes the racemization of D-Ser, D-Ala, D-homo-Ser, D-2-aminobutyric acid, D-Glu, and D-Asp.[241]

Isobutyl chloroformate (IBCF) is a well-known coupling agent in peptide synthesis and has been found to be a reagent of choice for large scale syntheses. One of the

drawbacks with this methodology is a side reaction, in some cases leading to the formation of urethane by-product and liberation of the starting amino acid. A case study on the elucidation of mechanism of urethane by-product formation and starting amino acid liberation during the conventional two-step isobutyl chloroformate (IBCF) mediated *N*-acylation is described using carbon dioxide offgas as the probe. The main reason for the urethane formation and starting amino acid liberation was found to be the formation of the symmetrical anhydride of the amino acid during the preparation of the mixed carboxylic-carbonic anhydride intermediate (Scheme 42), as determined by quantifying the evolved carbon dioxide. New conditions were developed to minimize this side reaction.[242]



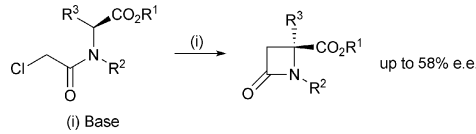
(i) IBCF, (ii) $-CO_2$, (iii) $C_6H_5–CH_2NH–CH_3$

Scheme 42

5.2 Reactions of amino acids

In recent years some chemical transformations have proceeded in a selective manner without using any external chiral source, a phenomenon that has been explained in terms of a new asymmetric concept known as 'memory of chirality'. Most of these transformations are found in the chemistry of α-amino acids. The enantioselectivity of the base-promoted cyclization of *N*-alkyl-*N*-chloroacetyl amino acid derivatives to β-lactams (Scheme 43) is dependent on the substituents on the starting material. Among these substituents, the amino acid side-chain (R^3) appears the principal stereodirecting element, offering additional support for the explanation that the memory of chirality is caused by a hindered rotation around the C–N bond.[243]

Reactive nitrogen species such as peroxynitrite and nitrogen dioxide have been implicated in the etiology of diverse pathophysiological conditions such as



Scheme 43

inflammation, neurodegenerative diseases, cardiovascular disorders, and cancer. Among the various amino acids in proteins, Trp is one of the most reactive and especially susceptible to attack by reactive nitrogen species such as peroxynitrite and nitrogen dioxide. Such loss of Trp residues in proteins has been associated with functional impairment of several proteins such as human Cu,Zn-superoxide dismutase *in vitro*. As shown in a study, Trp residues in proteins can be nitrated, nitrosated, and oxidized by reactive nitrogen species.[244] The use of ruthenium complexes as nitric oxide scavengers is one therapeutic approach for attenuating nitric oxide-mediated diseases. If a transition metal complex has a high affinity for the binding of nitric oxide, combined with low toxicity and favourable pharmacokinetics, it could be used as a treatment for these diseases. A remarkable discovery was that treatment of $[Ru(\beta\text{-diketonato})_2(MeCN)_2][CF_3SO_3]$ with an amino acid dithiocarbamate resulted in CS_2-elimination to yield a chelated amino (or imino) acidato complex. The amino acidato complexes were isolated from the reactions with *N*-methylisoleucinedithiocarbamic acid. This finding may be useful for the synthesis of other chelated amino (or imino) acid Ru(III).[221]

Among the numerous methods available for C–C bond formation, the enantioselective addition of organozinc to aldehydes appears to be one of the most useful methods. Natural amino acids form one of the best and the cheapest available chiral pools and many chiral ligands have been developed from them, *e.g.* β-amino alcohols were easily prepared from L-Phe in three simple straightforward steps. The effect of the substitutents on the nitrogen atom in piperidine-based amino alcohols was compared with similar pyrrolidine-based ligands.[245] Catalytic reduction of Phe and phenyl-Gly derivatives can be achieved with rhodium on carbon or alumina to give good yields of the corresponding cyclohexyl derivatives. The procedure can be scaled.[246]

Non-enzymatic glycation, an early stage of the Maillard reaction is one of the post-translation modification processes between free reducing sugars and free amino groups of proteins. This process is initiated by the condensation of reducing sugars and free amino group to form Schiff bases, which undergo rearrangements. Methylglyoxal (MG) is an endogenous metabolite, which is elevated in diabetics, and which reacts with amino acids to form advanced glycation end products. It was shown that the MG/lysine/Fe^{3+} reaction may lead to oxidative DNA damage. Deoxyribose assays showed that hydroxyl radicals were generated during the MG/Lys/Fe^{3+} reaction. These results suggest that the superoxide anion and H_2O_2 may be generated from the glycation reaction between Lys and MG, and that the Fe^{3+} probably participates in a Fenton's type reaction to produce hydroxyl radicals, which may cause DNA cleavage.[247]

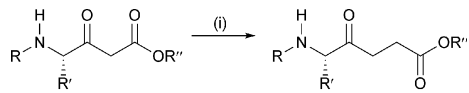
Photocatalytic reactions occurring at semiconductor particles/solution interfaces can be applied to organic syntheses. In a review article, examples of photocatalytic syntheses of cyclic amino acids by suspended semiconductor particles, *e.g.*, titanium(IV) oxide or cadmium(II) sulfide in aqueous suspensions are introduced and interpreted.[248]

5.3 Specific reactions of amino acids

Utilization of isosteric replacements for peptide bonds has been an attractive strategy for enzyme inhibition. Successful inhibition of aspartic acid protease targets

like HIV-protease and renin has validated the use of ketomethylene and hydroxyethylene isosteres in peptide systems. The availability of a simple, efficient reaction that facilitates the preparation of heavily functionalized, amino acid-derived γ-keto esters would find utility in the preparation of these isosteres, regardless of the inhibition target. Ketomethylene isosteric replacements for peptide bonds have been generated through a zinc carbenoid-mediated chain extension reaction in which a variety of amino acid-derived β-keto esters are converted to γ-keto esters in a single step (Scheme 44). The reaction tolerates a variety of protecting groups and amino acid side chains with no epimerization of the amino acid stereocenter.[249]

Recent discoveries and new applications in hydantoinase biocatalysis have been summarized with particular attention to stereoselective reactions and new mechanistic understanding of stereocontrol as well as new enantiopure products and novel applications.[250] An electrochemical oxidation of optically active *N*-acylated α-amino acids gave *N*,*O*-acetals. The nature of the protecting groups, reaction conditions, and anodic materials were found to affect the enantioselectivity.[89]



(i) Et_2Zn, CH_2I_2

Scheme 44

6 Analytical methods

6.1 Gas-liquid chromatography

Liquid chromatography using chiral stationary phases (CSPs) is one of the most sophisticated means of separating enantiomers and determining their composition. Among CSPs that form diastereomeric associations between enantiomers, hydrogen bonding is the most significant contributor to the associations. Enantiomer separation has been described by aqueous liquid chromatography using CSPs in which temperature-responsive polymers derived from acryloyl-L-valine *N*-methylamide and its *N*,*N*-dimethylamide analogue were bound on silica gel supports. During chromatography, enantioselectivity and retentivity for solute enantiomers were controlled by column temperature, which changes the aggregation and extension states of the chiral polymers depending upon their interior hydrophobic nature.[251]

Diseases involving the loss or breakdown of bone matrix represent a major healthcare problem. The amino acid sequence of collagen, the major protein component in bone, is rich in proline. Approximately 50% of the proline side chains are hydroxylated post-translationally to form hydroxyproline (Hyp). During collagen breakdown, Hyp is released from bone and not recycled to form new collagen. Serum or urinary Hyp is therefore considered to be an osteoclastic bone resorption marker. A sensitive and selective gas chromatographic-mass spectrometric (GC-MS) assay is an appropriate method for quantitation of hydroxyproline in bone. It replaces a combination of derivatization procedures by a simpler one-step silylation procedure. The employment of an anion-exchange resin effectively extracted and cleaned the sample and enhanced the assay selectivity.[252]

Gas chromatographic methods have some advantages over HPLC, but derivatization of amino acids needs to be performed before analysis to produce volatile compounds. Despite the remarkable achievements in separation and detection of amino acids, sample preparation before analysis remains an integral and often time consuming part of the methodology. A new derivatization–extraction method which avoids tedious preconcentration steps has been established in order to determine amino acids accurately at nanogram levels. The method involves conversion of the

analytes to *N*(*O*,*S*)-ethoxycarbonyl amino acid ethyl esters and subsequent extraction by single-drop microextraction SDME followed by GC analysis.[253]

The determination of D- and L-Asp content from teeth has been applied in age estimation for forensic purposes over the past decades. The application of the internal standard method has been investigated to determine age from Asp racemization in root dentin. This method compensates for uncertainties resulting from sample derivatization or preparation. D-Met and D-Nle were tested as internal standards for the purpose of validating the derivatization and gas chromatographic measurements. High accuracy in age determination along with the quality assurance provided by the internal standard method was observed.[254]

A chiral diamide stationary phase with different length of spacers has been synthesized by chemically bonding the *N*-(3,5-dimethylbenzoyl)-D-phenyl-Gly on the aminopropylsilica gel or silica gel bonded with different carbon chain length of spacers. These CSPs have successfully been used for the chiral resolutions of benzoyl-DL-amino acid isopropyl esters in normal-phase liquid chromatography (NP-LC). The introduction of secondary amine group in the spacer leads to the decline of separation factors. The effect of organic modifiers has also been examined.[255]

6.2 Ion exchange chromatography

Today, ion-exchange, apart from reversed-phase chromatography is the most popular separation method in liquid chromatography. During the last few decades, ion-exchange has become a purification method in preparative or production scale. When ion-exchange chromatography is used for separation of amino acids, fixed-bed adsorber is necessary for adequate prediction of the breakthrough curve. A mathematical model has been presented for both fluid and porous phases. A hybrid approach for solving the model for fixed-bed biosorption was proposed which has the advantage of being easier to apply than the purely analytical solution. The results from the model are compared with those obtained experimentally using Phe and Tyr diluted in aqueous solutions in a fixed bed of (poly-4-vinylpyridine) resin (PVP). Although the mathematical model has some limitations, it can be applied to a variety of situations.[256] A theoretical framework for the ion-exchange behaviour of bioactive substances in non-linear ion-exchange chromatography was studied and created a model basis to support a process design for production-scale ion-exchange chromatography. The theory can be applied to a whole variety of biological substances including amino acids.[257]

An isocratic chromatographic separation with amperometric detection of underivatized amino acids at a copper oxyhydroxide modified glassy carbon electrode has been described. A simple and sensitive quantification procedure of amino acids without the need of tedious and time-consuming derivatization step was achieved by coupling anion-exchange chromatography with electrochemical detection.[258]

Since most amino acids are poor chromophores, high concentrations are required for detection by ultraviolet absorbance. Anion-exchange (AE) chromatography using sodium hydroxide (NaOH) and sodium acetate gradients, coupled with integrated pulsed amperometric detection (IPAD), determines amino acids without sample derivatization. It was shown that AE-IPAD can be used to simultaneously determine amino acids and carbohydrates in cell culture. High precision, method ruggedness, and good spike recovery were observed using the optimized method.[259] New off-line sample preparation has been introduced that eliminates carbohydrates from amino acid samples containing a high carbohydrate content before analysis by anion-exchange chromatography and IPAD. All amino acids were recovered following the carbohydrate removal step.[260]

Disposable thin-film gold working electrodes have been prepared on polymeric substrates. This new type of electrode has been used in flow-through electrochemical cells to replace the conventional non-disposable gold working electrodes for integrated pulsed amperometric detection (IPAD) of compounds separated by

high-performance cation-exchange chromatography. Using two S-containing amino acids (homocysteine and Cys) as test compounds, a previously reported waveform has been modified for optimum performance with disposable gold electrodes.[132] Following separation by ion chromatography (IC), an electrode modified with a multiwall carbon nanotubes (MWNTs) film was used as an amperometric sensor for the simultaneous determination of oxidizable amino acids including Cys, Trp, and Tyr. The electrochemical behaviors of these amino acids at this modified electrode were studied by cyclic voltammetry (CV). The results indicated that the MWNTs chemically modified electrode (CME) exhibited efficient electrocatalytic activity towards the oxidation of these amino acids with relatively high sensitivity, stability, and long-life.[261]

6.3 Thin-Layer Chromatography (TLC) and related separation methods

Separation of amino acids and identification of the components of their mixture are frequent tasks in biochemistry. Considering that they are non-volatile compounds, TLC comes to mind first as a fast, simple, and inexpensive approach to reach this goal. However, since most of these compounds are UV-inactive (without derivatization), instead of the standard UV detection some methods of vibrational spectroscopic detection and identification should be applied. During the last two decades, there has been a continued interest in application and development of separation techniques coupled with spectroscopic detection. Identification of the separated components of a mixture by purely chromatographic characteristics, *i.e.* retention times in liquid (LC) and gas chromatography (GC), or retardation factors in TLC, cannot be done unequivocally, thus the demand for spectroscopic fingerprinting in order to increase the reliability of the analysis is highly justified. Raman spectroscopy using a (focused) laser beam for excitation may seem to be ideally suited for *in situ* measurement of small size analyte spots on TLC plates. Surface enhanced Raman spectra of TLC spots of amino acids could be generated by adding Ag-sol on top of the analyte spot and keeping the sample wet during the measurement. Interaction with Ag particles introduces frequency shifts and selective enhancement of various bands, which poses further difficulties in analyte identification. A comparative study of the feasibility and efficiency of Raman spectroscopic detection of thin layer chromatography (TLC) spots of some weak Raman scatterers (essential amino acids, namely, Gly and L-forms of Ala, Ser, Val, Pro, hydroxyproline, and Phe) was carried out using four different visible and near-infrared (NIR) laser radiations.[236]

Ninhydrin is a well-known, widely used chemical for colorimetric determination of amino acids. The colorimetric determination of amino acids and proteins using ninhydrin has some shortcomings, *e.g.* the colored amino acid spots are short-lived and disappear in a day, and the ninhydrin reagent is toxic. Geniposide and genipin have been isolated from gardenia fruit. Geniposide can be hydrolyzed by β-glucosidase to genipin, which in turn reacts with amino acids to form blue products. The colorimetric detection of amino acids using this genipin reaction was evaluated and compared with the well-known ninhydrin reaction. TLC analysis showed that the genipin reaction produces clear and stable colored spots.[262]

6.4 High-Performance Liquid Chromatography (HPLC)

A literature overview has been presented of chromatographic methods currently in use to determine amino acids and amines simultaneously or amines alone subsequent to their isolation from amino acids. Separation, derivatization and chromatographic conditions are also summarized. Advantages and drawbacks of all three possibilities are discussed and criticized in detail.[263]

Use of a quinine-derived chiral anion-exchanger stationary phase for the direct separation of *N*-protected unusual α-substituted Pro analogues by HPLC has been

reported. The chromatographic conditions were varied to achieve optimal separation. The readily prepared 2,4-dinitrophenyl derivatives were well separable, with good efficiency and high resolution. The method also proved suitable for determination of the enantiomeric purity of Pro analogues obtained by asymmetric synthesis.[264] Two macrocyclic antibiotic type chiral stationary phases (CSPs), based on native teicoplanin and teicoplanin aglycone, Chirobiotic T and Chirobiotic TAG, respectively, were evaluated for the HPLC separation of enantiomers of 15 unnatural conformationally constrained α-amino acids, Phe and Tyr analogs, and 12 β-amino acids having cycloalkane or cycloalkene skeletons. It is clearly established that in most cases the aglycone is responsible for the enantioseparation of amino acids. By application of these two CSPs excellent resolutions were achieved for most of the investigated compounds by using reversed phase or polar organic mobile mode systems.[265]

A chromatographic method for the separation and determination of D- and L-thyroxine enantiomers (D-, and L-T4) in human serum with a chiral ligand ion-exchange system using a chiral mobile phase additive and a silica column was established. An aqueous eluent containing L-Pro sufficiently complexed copper II ions and triethylamine (TEA) was used. It was monitored with a UV detector. The method has acceptable sensitivity, precision and accuracy for analysis. The concentration of D-T4 and L-T4 in serum of thyroid patients was determined by HPLC with a chiral additive and monitored with a UV detector.[266]

Stable 3-nitro Tyr (3-NO_2-Tyr), *o*-, *m*-, and *p*-Tyr isomers induced by oxidation of Tyr residues in protein were considered important biomarkers for the existence of toxic oxidizing agents, which could lead to diseases. Fluorescence detection is highly sensitive to *o*-, *m*-, and *p*-tyrosine, but it cannot be used to detect 3-NO_2-Tyr, due to the strong fluorescence-quenching characteristic of the NO_2 group. A highly sensitive reversed phase HPLC-UV method has been developed which was combined with pre-column cloud point extraction (CPE) to simultaneously determine *o*-, *m*-, and *p*-Tyr and 3-NO_2-Tyr. The combination of these two procedures contributed to the high sensitivity of this method. The sensitivity was significantly improved in comparison to that of previously developed HPLC methods that used direct UV detection and even in comparison to fluorescence detection methods.[267]

An important component of an analytical method is the ability to produce a signal that is free from all interferences. Due to the complex matrices that are often encountered, analytical methods for amino acids rely heavily on separations using liquid chromatography or capillary electrophoresis. In addition, extraction and derivatisation are often required to achieve optimum selectivity and sensitivity. Such methodologies do not lend themselves to rapid analysis. Chemiluminescence has been shown to be a sensitive and selective detection mode for flow injection analysis (FIA), and has been utilised for the direct determination of a wide range of analytes in complex matrices without prior separation. The selective determination of the following amino acids has been described using flow injection analysis (FIA) with chemiluminescence detection: Pro, His, Tyr, Arg, Phe, and Trp.[268] This approach offers significant advantages over conventional methods since derivatisation, separation, or extractions are not required.

Nitric oxide (NO) along with citrulline is synthesized by nitric oxide synthase (NOS) from endogenous or dietary supplemental L-Arg. Many methods have been developed for the separation and determination of L-Arg and dimethylarginines in biological samples. These methods include HPLC, capillary zone electrophoresis (CZE), and LC-tandem MS. Among these methods, HPLC might be the most useful one. The *o*-phthalaldehyde (OPA) pre-column fluorescence derivatization technique is used in all the reported HPLC methods to increase the detectability. A simple, sensitive and fast method using RP-HPLC—MS coupling with an atmospheric pressure chemical ionization interface was developed for simultaneous separation and determination of L-Arg and its N^G,N'^G-dimethyl derivatives.[269]

A novel optically active thiol compound, *N*-(*tert*-butylthiocarbamoyl)-L-cysteine ethyl ester (BTCC), has been synthesized as a chiral derivatization reagent. This compound and *o*-phthalaldehyde react with amino acid enantiomers to produce fluorescent diastereomers that are readily separable on a reverse-phase column by HPLC. Enantioseparation of acidic amino acids *e.g.* Asp is markedly improved using BTCC. Since this method is rapid and fully automated, it is additionally suitable for assaying large numbers of samples.[270] A new method has been developed for the sensitive determination of amino acids and peptides using the sensitive fluorescent reagent 2-(9-carbazole)-ethyl chloroformate (CEOC). Identification of derivatives was carried out by reverse phase HPLC and liquid chromotography mass spectrometry (LC-MS).[271]

It has been suggested that the plasma concentration of D-Ala increases in cases of several disease. For the determination of D-amino acids, a variety of enzymatic methods and chromatographic methods including gas chromatography (GC), liquid chromatography (LC), HPLC in combination with various derivatizing reagents, and capillary electrophoresis (CE) have been reported. A column-switching chiral HPLC system for the determination of D-Ala in mammalian tissues has been established.[272]

A variety of α-amino acids were enantioresolved for the first time on naphthylethylcarbamate-β-cyclodextrin bonded phases (*i.e.*, SN- and RN-β-CD) using the acetonitrile-based mobile phase after their pre-column derivatization with phenyl isothiocyanate in alkaline medium. The enhanced resolution on RN-beta-CD phase was believed to be due to the re-location of the hydrogen receptor site from sulfur to nitrogen on the isothiocyanyl fragment of derivatizing reagent, which in turn changes the enantioselectivity. Also, the sulfur atom is larger in size and subject to steric hindrance more significantly in comparison with oxygen. The position of the amino group on the backbone affects the resolution.[273]

Addition of hydroxypropyl-β-cyclodextrin to *o*-phthalaldehyde (OPA)–amino acid-thiol reaction mixtures has been shown to cause significant enhancement of the fluorescence of the isoindole product for a wide range of amino acids. The significant fluorescence enhancements and very large derivative stabilization observed suggest that addition of hydroxypropyl-β-cyclodextrin would be beneficial to OPA-based HPLC or HPCE techniques, either pre- or postcolumn, for detecting and measuring amino acids[274] Some factors influencing the separation and detection of amino acids by high-performance anion-exchange chromatography with integrated pulsed amperometric detection were investigated. The optimized gradient elution condition and column temperature for analyzing 19 amino acids were obtained.[275]

In recent years, cyanuric chloride (2,4,6-trichloro-1,3,5-triazine; trichloro-*s*-triazine) attracted increasing attention for potential use in chromatography. This is attributed to its high reactivity and trifunctionality that allows easy and controlled sequential replacement of the halogens by nucleophiles. A series of chiral derivatizing reagents (CDRs) was synthesized by nucleophilic replacement of one chlorine atom in cyanuric chloride (2,4,6-trichloro-1,3,5-triazine; *s*-triazine) by alkoxy (methoxy, butoxy, 1,1,1-trifluoroethoxy) or aryloxy groups (phenoxy, nitrophenoxy, phenylphenoxy, 4-methylcoumaryloxy), and displacement of a second chlorine by L-Ala amide, L-Phe amide, L-Pro *tert*.-butyl ester, or Boc-L-Lys *tert*.-butyl ester. Further, CDRs were investigated in which two chlorine atoms in cyanuric chloride were substituted consecutively by L-Val amide and L-Phe amide. The resulting CDRs having a remaining reactive chlorine were tested for their capability of derivatizing DL-amino acids followed by liquid chromatographic separation of the resulting diastereomers.[191]

One of the most popular techniques for the analysis and direct separation of enantiomers is HPLC utilizing a chiral stationary phase (CSP). The effect of the mobile phase parameters, flow rate, temperature, pH and ionic strength, as well as the addition of various organic modifiers on the enantiomer separation of various

aromatic α-amino acids was investigated using two antibody-based chiral stationary phases that have opposing stereoselectivity. The addition of organic modifiers did not improve separations.[276] A new method for simultaneous analysis of amino acids and biogenic polyamines after pre-column derivatization with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu) has been proposed. The described combination of columns and mobile phase significantly reduces the individual analysis costs, shortening the time of analysis, lowering the organic solvent consumption and extending the column life.[277] Another method for simultaneous quantitation of amino acids and common polyamines was developed and protocols were optimized for dansyl derivatization.[278]

6.5 Mass Spectrometry (MS)

The importance of monitoring amino acids and peptides in biological processes has encouraged the expansion of analytical methodologies. In most cases, selective detection of these compounds is achieved only after attaching some type of label to the amino group. Increased interest in amino acid and peptide analysis has led to the introduction of a wide range of agents for labeling amines, including ninhydrin, fluorescamine, dansyl chloride, 7-fluoro-4-nitrobenz-2,1,3-oxodiazole (NBD), *o*-phthalaldehyde (OPA), and naphthalene-2,3-dicarboxaldehyde (NDA). The requirements of the derivatization reaction and the stability of the derivative are deciding factors in choosing a derivative. Therefore, in addition to optimizing derivatization parameters, a thorough understanding of factors influencing derivative stability is critical. In this study, the stability of amino acids derivatized with NDA was investigated using a combination of HPLC, solid-phase extraction, photodiode array spectrophotometric detection, and mass spectrometric (MS) characterization. Tandem mass spectrometry (MS/MS) experiments were used to demonstrate unimolecular degradation of the protonated isoindole in the absence of solvent or atmosphere, suggesting an intramolecular reaction mechanism involving the hydroxyethylthio group. The results suggest that the degradation pathway for NDA derivatives is similar to the previously reported pathway for *o*-phthalaldehyde derivatives and clearly identifies the reaction and degradation products under a variety of conditions.[279]

A matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)-based method has been developed for the quantitative analysis of amino acids. This method does not require any amino acid modification, derivatization, or chromatographic separation. The data acquisition time is decreased to several seconds for a single sample due to the absence of modification and separation steps. No significant ion suppression effects were observed with the developed sample deposition technique. The method was found to be reproducible and ratio of the peak intensities of corresponding amino acid to internal standard were observed for all amino acids analyzed. This method was applicable to the mixtures of free amino acids, to HCl hydrolysates of proteins, as well as to other biologically important low-molecular weight compounds.[280] Coupling of HPLC with MS has been widely explored for analyzing post-translational modification of protein-derived peptides or amino acids to facilitate structural identification on the basis of mass. A practical synthesis has been described for *N*,*N*-dimethyl-2,4-dinitro-5-fluorobenzylamine (DMDNFB) and its -d_6 analog as an alternative Sanger's reagent (DNFB), for purposes of amino acid derivatization detectable by positive mode electrospray ionization(ESI) MS.[281]

Several chromatographic methods, including liquid chromatography (LC), gas chromatography (GC), GC-MS, have been employed to analyze levels of metabolites in biological samples. GC-MS technology allows simultaneous detection of different classes of metabolites within a single analysis, but it requires the conversion of these compounds to volatile derivatives. Some disadvantages with the popular derivatization technique are silylation under anhydrous conditions, heating of the sample for a given period, and injection of the reactive mixture onto the

chromatographic column. A new approach to the derivatization of molecules containing carboxylic groups resulted from an investigation of the alkyl chloroformates (CFs), which are able to produce mixed anhydrides. The main advantages of these derivatizing agents are (i) a very fast reaction without requirement for sample heating or exclusion of water, (ii) negligible reagent cost, and (iii) a simple sample workup that makes it unnecessary to isolate the analytes from the matrix. The methods can easily be automated through the use of commercially available analytical robots. A new protocol has been presented which is based on CF derivatives for derivatization of amino acids simultaneously with di- and tricarboxylic acids.[282]

The LC-ESI-MS methods can be applied for the separation and identification of isomers of proteinogenic and unusual amino acids, especially those that are of high interest in biomedical research. The separation and stereochemical assignment of a variety of unusual amino acids such as β-methoxy-Tyr, *allo*-configured and/or *N*-methylated forms of Thr, Ile and Phe has been described by reversed-phase high-performance liquid chromatography—electrospray ionization mass spectrometry (RP-HPLC—ESI-MS). Indirect chromatography was carried out after precolumn derivatization and subsequent separation of the resulting diastereomers of each amino acid was monitored by UV and MS. The strengths and limitations of different chiral derivatizing agents are also discussed in detail.[283]

The oxidation of proteins by free radicals plays a major role in many oxidative processes within cells. The oxidized forms of proteins accumulate during aging, oxidative stress, and several pathological conditions. Hydroxyl radical is known to be one of the most reactive oxygene species (ROS) *in vitro* and *in vivo* systems, capable of reacting with almost all constituents of the cell. All amino acid residues are susceptible to oxidation by hydroxyl radical. After the oxidation of Trp, using hydrogen peroxide and iron (II) system (Fenton reaction), new oxidation products and free radicals were identified using mass spectrometry (ES-MS) and ES-MS/MS.[284] Besides mono- and dihydroxy tryptophans and *N*-formylkynurenine, new products resulting from the reaction of tryptophan and oxidized tryptophan and 3-methyl indole derivatives were also identified. Tandem MS was also used to confirm the proposed structure of the observed adducts. The L-Arg/NO biosynthetic pathway is involved in many physiological and pathophysiological processes. Asymmetric dimethyl-Arg (ADMA; N^G,N^G-dimethyl-L-Arg) is the most important endogenous inhibitor of nitric oxide synthase. A GC—tandem MS method for the accurate quantification of ADMA in human plasma or serum and urine has been reported using *de novo* synthesized [$^2H_3$]-methyl ester ADMA (d_3Me-ADMA) as the internal standard.[285]

Chiral recognition has long been an area of considerable interest in MS. The challenge has been that both enantiomers have identical masses and physical characteristics under mass spectrometry. Enantiomers differ primarily by how they interact with other chiral compounds (selectors). In this regard, chiral differentiation methods that employ mass spectrometry are akin to the chromatographic method where the analyte is allowed to interact with a chiral selector. In mass spectrometry, the nature of these interactions can be made to manifest themselves under various ionization and ion dissociation conditions. The advantage of mass spectrometry, however, is speed, sensitivity, and structural information. The properties of host–guest complexes involving the analyte (guest) and the selector (host) have been examined for their potential utility for chiral analysis. The gas-phase guest exchange reactions of a number of non-natural α-amino acids complexed to permethylated β-cyclodextrin were examined with Fourier transform mass spectrometry. The enantioselectivity of the reactions were determined. Molecular modeling calculations were performed to support the experimental results. The amino acids included homoSer, *cis*-4-hydroxy-Pro, allo-Thr, and allo-Ile. Results from calculations of molecular modeling suggest that enantioselectivity is governed by differences in the binding interaction between the amino acid host and the permethylated β-cyclodextrin guest.[286]

Sodium ion is one of the most abundant metal ions in biological systems, where it is involved in a variety of processes, including osmotic balance, the stabilization of biomolecular conformations and information transfer *via* ion pumps and ion channels. Na^+-bound heterodimers of amino acids (AA) are produced in the gas phase by electrospray ionization (ESI). The dissociation kinetics of these AA_1–Na^+–AA_2 ions were determined by collisionally activated dissociation (CAD) and converted to a ladder of relative Na^+ affinities *via* the Cooks kinetic method. Experimental and *ab initio* affinities agree very well. The combined data show that functional side chains increase the AA–Na^+ bond strength by providing an extra ligand to the metal ion. A poor correlation is found between sodium ion and proton affinities, strongly suggesting that the Na^+ complexes do not have salt-bridge structures involving zwitterionic amino acids (in which the most basic site is protonated).[287]

The results of capillary electrophoresis dynamic reaction cell™ inductively coupled plasma mass spectrometry (CE-DRC-ICP-MS) for the determination of sulfur-containing amino acids demonstrated that the concentrations of sulfur-containing amino acids in samples could be determined by CE-DRC-ICP-MS without complicated sample pretreatment.[288] Protein footprinting utilizing hydroxyl radicals coupled with mass spectrometry has become a powerful technique for mapping the solvent accessible surface of proteins and examining protein–protein interactions in solution. Hydroxyl radicals generated by radiolysis or chemical methods efficiently react with many amino acid residue side chains, including the aromatic and sulfur-containing residues along with proline and leucine, generating stable oxidation products that are valuable probes for examining protein structure. The radiolytic oxidation chemistry of histidine, lysine, and arginine has been investigated for comparison with their metal-catalyzed oxidation products. All three basic amino acid residues are susceptible to radiolytic oxidization by γ-rays or synchrotron X-rays. These residues, particularly Arg and Lys, are preferentially located at protein surfaces and are frequently involved in protein interactions. It was demonstrated how these residues can be utilized in future footprinting experiments to probe protein structure.[289]

6.6 Capillary Electrophoresis (CE)

Capillary electrophoresis (CE) separation is based on differences in the electrophoretic mobility of the analytes. It is a powerful technique and has been used to separate a large variety of charged species. CE shows many advantages over more traditional chromatographic methods for ionic analytes, however, one disadvantage of CE is the lack of a convenient means to vary the separation selectivity of the system. This drawback can be overcome by the use of pseudostationary phases (p-SPs) in the background electrolyte that can interact with the analytes of interest and greatly increases the selectivity control. Capillary electrochromatography (CEC) is a rapidly growing area in separation science. The prevailing extraordinary level of theoretical and practical interests in CEC is explained by the fact that CEC effectively combines advantages of two major separation techniques: capillary zone electrophoresis (CZE) providing high separation efficiency and HPLC. CEC also provides efficient separations for both charged and uncharged molecules. The most important applications in capillary electrochromatography (CEC) have been reviewed. A selection of new developments in stationary phases for CEC is highlighted, and enantiomeric separations and chiral stationary phases are discussed. Also, CEC applications of biological molecules, pharmaceuticals, and in the field of industrial and environmental analysis are summarized. For this review three modes of CEC were taken into account, *i.e.*, packed-column CEC, CEC using monolith technology, and open-tubular CEC.[290]

In a recent study, advances in amino acid analysis by CE have been reviewed. Detection methods, separations of enantiomers, the new medical applications, and amino acids in food and plants were discussed.[291] A non-aqueous CE method was

developed for enantiomer separation of *N*-protected (benzoyl, 3,5-dinitrobenzoyl, and 3,5-dinitrobenzyloxycarbonyl) amino acids by non-aqueous CE and HPLC with *tert*.-butyl carbamoylated derivatives of quinine and quinidine in either the background electrolyte or the stationary phase.[292]

Chiral separation using sol–gel stationary phases is a rapidly growing area in CEC. Sol–gel stationary phases have been primarily used for the separation of small molecules, although properly designed sol–gel stationary phases should have great potential in the separation biological macromolecules as well. The advances made in the area of scientific research devoted to the design, synthesis, characterization, and application of sol–gel stationary phases for various CEC column formats have been summarized.[293] CEC separations of amino acid mixtures were studied using two modified porous photopolymerized sol–gel monolithic columns. One was modified with dimethyloctadecylchlorosilane (DMOS), and the other was modified with DMOS, followed by chlorotrimethylsilane to end-cap residual silanol groups.[294] A monolithic silica column was fabricated inside a fused-silica capillary with 100 μm inner diameter by sol–gel process. Gamma- and beta-CD-modified monolithic column has successfully been applied for the separation of dansyl amino acid enantiomers and and the enantioseparation of racemates of benzoin and several dansyl amino acids by capillary electrochromatography, respectively.[295]

Enantioselective ligand exchange in combination with electromigration separation techniques and chromatographic techniques, as well as in liquid–liquid partitioning systems, capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC), enantiomeric separations on chiral stationary phases (CSP), chiral coated phases (CCP) in ligand exchange chromatography, and chiral mobile phase (CMP)-type separations have also been discussed.[296] The potential of *N*,*N*-dimethylacrylamide-piperazine diacrylamide-based monolithic stationary phases bearing sulfonic acid groups for electroosmotic flow generation was investigated for the separation of positively charged amino acids and peptides. The capillary columns were used under electrochromatographic but also under purely chromatographic (nano-HPLC) conditions and the separations interpreted as the result of possible chromatographic and electrophoretic contributions. The monolithic column was capable of supporting both electrostatic and hydrophilic interactions. An ion-exchange mode superposed with electrokinetic migration was found to be the dominant mode.[297]

An automatic, rapid and continuous on-line derivatization system coupled to microfluidic CE for the determination of amino acids using *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) as the derivative agents has been developed. By on-line derivatization, amino acids were automatically and reproducibly converted to the UV-absorbing derivatives, which were separated by capillary zone electrophoresis (CZE).[298] Chiral separations of fluorescamine-labeled amino acids were characterized and optimized on a microfabricated capillary electrophoresis (CE) device. A standard mixture of acidic and neutral amino acids were labeled with fluorescamine. The results demonstrate the feasibility of combining fluorescamine labeling of amino acids with microfabricated CE devices to develop low-volume, high-sensitivity apparatus[150] A method based on microchip CE with amperometric detection was developed for the rapid separation and direct detection of oxidizable aromatic amino acids without prior derivatization. The working electrode was a thick-film carbon strip electrode positioned opposite the outlet of the separation channel. Factors influencing the separation and detection processes were examined and optimized. The five aromatic amino acids, Tyr, 5-hydroxy-Trp, Trp, *p*-aminobenzoic acid, and *m*-aminobenzoic acid, can be well separated within 5 min. The high speed, negligible sample consumption and waste production, low power requirements and small size of the CE microchip device offer great promise for the determination of aromatic amino acids in clinical and biotechnological matrices.[299] Separations of amino acids, carbohydrates, and antibiotics by microchip electrophoresis with pulsed amperometric detection have been described. The new chip

configuration consists of a layer of poly(dimethylsiloxane) that contains the microfluidic channels, reservoirs, and a gold microwire, sealed to a second layer of poly(dimethylsiloxane).[300]

The CE method seems to be a good alternative for determination of amino acids and their analogues in multicomponent pharmaceuticals because of short analysis time and the possibility to assay all components during a single run without any pretreatment. The CE method allows the separation of all investigated analytes with high efficiency and enables a simultaneous analysis of amino acids and their keto and hydroxy analogues in tablets.[301] The CE dynamic reaction cell™ inductively coupled plasma mass spectrometry (CE-DRC-ICP-MS) for the determination of sulfur-containing amino acids (L-Cys, L-cystine, DL-homocystine and L-Met) has been described.[288]

A simple and reproducible method, based on micellar electrokinetic capillary chromatography (MECC) with amperometric detection (AD), for the separation and determination of biogenic amines and their precursor amino acids was investigated. The optimal conditions of separation and detection of tryptamine, tyramine, Trp, and Tyr were determined. Under the optimum conditions, the four analytes were separated completely within 15 minutes, and good reproducibility and recovery results were obtained.[302]

A novel chemiluminescence (CL) detection scheme has been developed for detecting underivatized amino acids following CE separation. This detection was based on the inhibitory effect of amino acids on the CL reaction between luminol and BrO^- in alkaline aqueous solution. Parameters affecting CE–CL separation and detection, such as the pH value, the concentration of electrolyte, and CL reagent on the resolution were optimized.[303] Twenty α-amino acids were directly detected by CE with a CL detector using luminol-hydrogen peroxide-Cu(II) system. A model mixture sample of Gly, Asp, and Glu was successfully separated and detected within 10 min.[304] Laser-induced fluorescence (LIF) coupled to CE is one of the most powerful detection techniques to achieve low detection limits for biological compounds. LIF detection after derivatization has been used to analyze a broad range of molecules such as amino acids, acids, amines, and thiols. Trp and Tyr were analyzed in cerebrospinal fluid by capillary electrophoresis and ''ball lens'' UV-pulsed laser-induced fluorescence detection.[305]

Ligand-exchange micellar electrokinetic capillary chromatography was used for the chiral resolution of underivatized and dansyl amino acid enantiomers simultaneously. The separation was achieved by chiral copper(II)–L-Val complexes incorporated in micelles of sodium dodecyl sulfate.[189] A simple technique—CZE with direct UV-detection—was used for the express analysis of Lys, Met, Thr, and cystine contents of samples.[306] An efficient, sensitive and rapid analysis of the amino acid neurotransmitters (Glu, Asp, GABA, and Gly) in the cerebral cortex of rats was developed by CE with laser-induced fluorescence detection and fluorescein isothiocyanate (FITC) derivatization. Different parameters which influenced derivatization and separation were optimized.[307] Recent developments in sample preparation (on-line chemical or chromatographic sample clean-up condensation methods, on-line electrophoretic concentration methods, and on-line sample filtration and clean-up with a semi-permeable membrane for various CE modes) for CE have been discussed.[308]

6.7 Other analytical methods

These days, when separation methods can provide high resolution of complex mixtures of almost every matrix, from gases to biological macromolecules, and detection limits down to femtograms or below, the whole advanced analytical process still can be wasted if an unsuitable sample preparation method has been employed before the sample reaches the chromatograph. With increasing demands on the analytical chemist to provide accurate and valid analytical measurements for

regulatory requirements, poor sample treatment or a badly prepared extract will invalidate the whole assay and even the most powerful separation method will not give a valid result. Yet sample preparation is often a neglected area, which over the years has received much less attention and research than the chromatographic separation or detection stages. The importance of sample preparation methods as the first stage in an analytical procedure is emphasised and examined in a review.[309]

The recent chromatographic literature has been summarized by Shepherd.[310] This review covers advances in separation methods that involve transition metal chemistry assists readers in finding key papers that illustrate techniques of chromatography that might be applicable. Covered topics include the standard separation of inorganic ions and metal complexes, CE methods (CE, CZE, MEKC), electrochemical detection in flow methods by $[Ru(bpy)_3]^{3+/2+}$ cycling in response to analytes, separations of metal complexes of interest to environmental and biomedical disciplines *via* size exclusion chromatography (SEC), and detection methods with ESI-MS. Advances in affinity chromatography in the separation of peptides and proteins are also discussed. Recent advances in understanding the mechanisms of chromatographic separations, and of the technique of polymer imprinting to produce selective recognition sites for metal ions and complexes are described.

For many years, amino acid analysis has been done routinely. The hydrolysis of samples is performed in evacuated tubes or under an inert atmosphere in order to prevent oxidative losses of some amino acids. The process of evacuating and sealing samples in glass tubes is time consuming and requires a great deal of expertise, which limited the utility of the method in many cases until commercial instruments such as the Pico Tag workstation (Waters Millipore Corp.) became available. A communication has described the construction and operation of a simple device used to eliminate oxygen from a Pico Tag vapor-phase acid hydrolysis vessel for amino acid analysis.[311] An introduction to the basic implementation, the historical development and some of the uses of Marfey's reagent, 1-fluoro-2,4-dinitrophenyl-5-L-Ala amide, a pre-column derivatizing reagent for the separation of enantiomeric isomers of amino acids and amine compounds have been summarized.[312]

The blue spots from genipin–amino acid reactions were unchanged even after 7 months, while colored spots from the ninhydrin reaction disappear fairly quickly on a TLC plate. The colorimetric detection of amino acids using this genipin reaction was evaluated and compared with the ninhydrin reaction.[262] Ninhydrin is reported to have toxic effects and is also known to promote tumor development in laboratory animals. In contrast, the gardenia blue pigments are very safe compounds. Ascorbic acid is a safe reducing agent. The use of a modified ninhydrin reagent using ascorbic acid instead of potassium cyanide was investigated for the photometric determination of amino acids.[313]

Methods that are used to achieve selectivity include derivatisation, extraction, and permeability differences, all of which can be time consuming, tedious, and expensive. The selective determination of a range of amino acids (Pro, His, Tyr, Arg, Phe, and Trp) using flow injection analysis with chemiluminescence detection has been described. Selectivity is achieved by the application of a number of chemiluminescence reactions and the manipulation of reaction conditions when two or more amino acids gave a response to a particular reaction. The ability to measure individual amino acids in the presence of other amino acids using this approach has also been demonstrated.[268]

Over recent years, the measurement of Ala has been a main topic for different purposes. Due to its importance, various methods have been developed for the estimation of Ala concentration in different samples. A novel combination of salicylate hydroxylase (SHL), L-Ala dehydrogenase (AlaDH), and pyruvate oxidase (PyOD) trienzyme membrane resulted in an Ala sensor with high performance characteristics. The use of this trienzyme system provides the determination of Ala with high reliability, sensitivity and reproducibility, which were absent in the conventional Ala sensor system.[314]

A simple, sensitive ion chromatographic–amperometric method has been developed to determine some oxidizable amino acids such as Cys, Trp, and Tyr. After functionalisation with carboxyl groups, the multiwall carbon nanotubes (MWNTs) film showed sensitive and stable electrocatalytic activity to the oxidizable amino acids. Compared with the existing procedures, the advantages of this method are that it is simple and convenient, and no valuable detector needed.[261] L-Pipecolic acid (L-PA) is an important biomedical marker for the diagnosis of peroxisomal disorders. Amperometric biosensors have been designed for the determination of L- and D-PA in serum samples. The advantage of using amperometric biosensors above chromatographic methods is the simplicity of the method, rapidity, and low price of analysis. Furthermore, amperometric biosensors combine the high enantioselectivity of the enzyme with the sensitivity of the amperometric transducer.[315]

Dispersion free reactive extraction of L-Phe as well as other zwitterionic amino acids have been successfully extracted from dilute solution in a hollow fiber (HF) membrane module.[316]

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Peptide synthesis

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1. Introduction

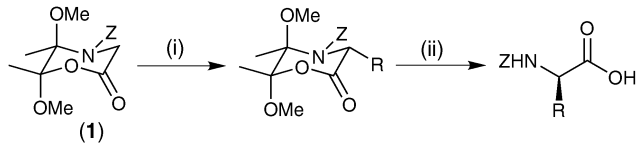
The present review covers the years 2003–2004, although the space permitted is the same as for previous reviews that covered one year. Consequently, some changes have had to be made to the format used in the previous Report[1] in order to meet this restriction. As in previous years, patents and meetings abstracts have not been included. In addition, no reviews have been cited. The section in previous reviews devoted to miscellaneous reactions of synthetic peptides (2.8) has been omitted but relevant references have been included in the appendix. Finally, work cited in the present review has been selected, so that no claim is made that the review is comprehensive. The present volume is the last to be published in this series by the Royal Society of Chemistry and it is hoped that the efforts of all the present and past Reporters have been helpful for researchers in this field.

2. Methods

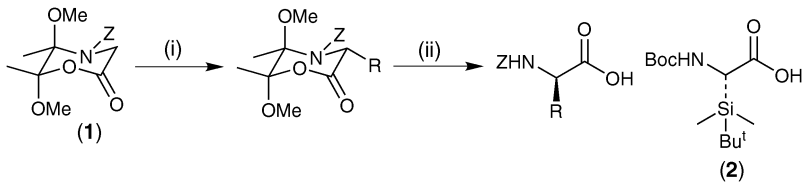
2.1 Amino-group protection

The use of the *N*-tetrachloro-phthaloyl group in SPPS has been examined.[2] Removal of the protecting group can be effected using either N_2H_4/$CHONMe_2$ (3:17) at 35 °C for 30 min or $(CH_2NH_2)_2$/$CHONMe_2$ (1:200) at 50 °C for 30 min. A report that maleimides are formed by the interaction of exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride and amines in aqueous solution has been refuted.[3] It is claimed that the true products are the hemimaleate salts of the amines. A glycine equivalent (**1**) has been synthesised from glycidol and converted into Z-amino acids after alkylation using lithium diisopropyl-amide and RX followed by hydrolysis[4] (Scheme 1). The Z-group can be removed by hydrogenation using a Pd catalyst on hydroxy-apatite.[5] The Z-group can also be removed from protected amino acids and 5-oxazolidinones using BCl_3 in $CHCl_3$ at room temperature.[6] Presumably this method would work with Z-peptides although this is not mentioned in the paper. The Z-group can also be removed enzymatically with a protein isolated from *Sphingomonas paucimobilis*.[7] Treatment of Z-derivatives with ZCl and lithium bis(trimethylsilylamide) at −78 °C affords the bis-Z derivatives.[8] Conversely, treatment of *NN*-Z_2- or *N*-Z,*N*-Tos derivatives with LiBr in MeCN removes a Z-group.[9] The synthetic importance of these last two techniques remains to be evaluated. High yields of *N*α-Z- and *N*α-Fmoc-Orn have been obtained by a route involving intermediate preparation of N^{δ}-Boc-Orn.[10] The introduction of Z- or Fmoc-groups can be achieved photochemically in neutral solution using the appropriate *N*-substituted 5,7-dinitroindolinines, but yields are low with sterically hindered amines and in preparing Boc derivatives.[11] Boc-amino acids can be prepared using $(Boc)_2O$ in the presence of a catalytic amount of $ZrCl_4$ in MeCN at room temprature. High yields are obtained in a few minutes.[12] Asymmetric aza-Brook rearrangement of *N*-Boc-*N*-trialkylsilylallylamine yields an enantiomerically enriched α-aminoallylsilane that undergoes oxidative cleavage to give an L-Boc amino acid (**2**).[13]

[a] *Eynsham, Witney, Oxfordshire UK*



Scheme 1 Reagents: (i) Pr_i^2NLi at −78 °C then RX; (ii) CF_3CO_2H/H_2O.



Fmoc-amino acids and Fmoc-dipeptides can be converted into Boc-derivatives while attached to Wang resin by a one-pot method using KF and either $(Boc)_2O$ or Bu^t-S-(4,6-dimethylpyrimidine-2-yl)thiolcarbonate.[14] Another method for generating Boc derivatives consists in reducing *N*-benzyl, *N*-trityl or *N*-diphenylmethyl derivatives with polymethylhydrosiloxane $\{Me_3SiO[MeHSiO]_nSiMe_3\}$ and treating with $(Boc)_2O$ under $Pd(OH)_2/C$ catalysis.[15] α-Diazoketones can be derived from Fmoc-α-amino acids by the Wolff rearrangement, a rapid process under microwave radiation giving high yields.[16] Fmoc-aminoketones, potential intermediates for the synthesis of proteinase inhibitors, are accessible in good yield from amino acids by transformation into thioesters of 2-mercaptopyridine and reaction of the latter with dialkylcuprates.[17] Alloc- and propargyloxycarbonyl-derivatives can be prepared using insoluble resins bearing the appropriately substituted *N*-hydroxysuccinimide group.[18] After use, the resin reagents can be recycled if desired. The 2-(2-nitrophenyl)-propyloxycarbonyl group is a new candidate for amino-group protection.[19] Deprotection is achieved by photolysis, a process that is faster than the removal of the previously described 2-nitroveratryloxycarbonyl group.

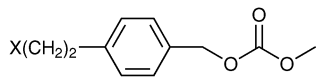
The use of the Dde group has not been widely favoured because deprotection has hitherto required the use of hydrazine which also removes the Fmoc group. It has now been discovered that the Dde group can be cleanly removed using hydroxylamine hydrochloride in *N*-methyl-2-pyrrolidone, conditions that leave the Fmoc group unaffected.[20] Consequently, the Fmoc and Dde groups are fully orthogonal. Protection of the amino group as the isopropylidene derivatives may be an alternative to the use of the Dde group, since it has been found the former are sensitive to treatment with ytterbium triflate and HOBt in $MeCN/CF_3CH_2OH$ (1:1) at 50 °C for 10 min.[21] In the special case where a cysteine residue is *N*-terminal, the amino and thiol groups can be protected by reaction with ninhydrin.[22] The resultant thiazolidine ring is stable during coupling reactions in solution or on solid phase or during treatment with acids. It is removed under thiolytic or reducing conditions. This technique may prove useful in native chemical ligation involving the coupling of several fragments. The use of Cu^{2+} ions to mask the α-amino and carboxy groups of lysine and ornithine in order to selectively protect the ω-amino group has been known for a long time. An alternative reagent is 9-borabicyclo(3:3:1)nonane and this has been used to operate selectively on the 5-hydroxy- and 6-amino groups of hydroxylysine;[23] the chelating group can be removed with ethylenediamine exposing the α-amino group to orthogonal protection if desired.

A few groups based on the β-sulfonylethyloxycarbonyl theme have been described. The simplest example is the (2-ethyl-sulfonyl)ethoxycarbonyl group.[24,25] A related group is the 2-(4-trifluoromethylphenyl)ethoxycarbonyl group.[26] This type of

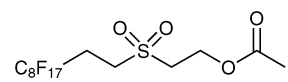
protecting group is labile to base whereas the related thioether is not. This forms the basis of the Kenner safety-catch mechanism. A water-soluble variant involves the replacement of the CF_3-group with a sulfonic acid group.[27] Another protecting group that guarantees water solubility of the product incorporates a sulfonium group.[28,29]

Polyfluorinated groups have become important since they assist in the chromatographic purification of peptide products. One such group (**3**) has apparently not been tested in peptide synthesis[30] and the derivatives of Lys, Orn and His have not been described. The other group (**4**) has been used in peptide synthesis and appears to help in purification.[31]

A final orphan contribution to this section reports the use of penicillin-G amidase for the *N*-acylation of D-amino acid esters in organic solvents.[32]



(**3**) X = C_6F_{13} or C_8F_{17}



(**4**)

2.2 Carboxy-group protection

The preparation of toluene-4-sulfonate salts of benzyl esters of amino acid is facilitated by carrying out the reaction of amino acid, benzyl alcohol and toluene-4-sulfonic acid under microwave irradiation.[33] The esterification of amino acids has also been conducted in ionic liquids, a technique that is rapidly gaining favour on environmental grounds.[34] Boc amino acids can be esterified in the appropriate alcohol in the presence of ceric ammonium nitrate. At room temperature, the Boc group survives, but is removed if the reaction is carried out under reflux.[35] Alkyl esters can be prepared by the interaction of an alkoxydiphenyl phosphine and the required acid in the presence of a mild quinone-type oxidant such as 2,6-dimethyl-1,4-benzoquinone.[36] Ethyl esters of *N*-protected amino acids have been prepared by reaction with 3-(ethoxycarbonyl)benzotriazole-1-oxide in EtOH/MeCN in the presence of Et_3N.[37] Presumably this method would be satisfactory for the preparation of other alkyl esters, but reassurance would be desirable that chiral purity is not impaired. A similar technique uses polystyrylsulfonyl-3-nitro-1H-1,2,4-triazolide resin, made from polystyrylsulfonyl chloride and 3-nitro-1H-1,2,4-triazole, as a catalyst for esterifying amino esters.[38] Racemisation was shown not to be a problem. Polymer-bound alkyltriazenes also esterified amino acids and peptides with concomitant loss of nitrogen and transfer of the alkyl group to the carboxy group to form the ester. Chiral purity was completely retained.[39] In contrast, phenacyl esters have gained in popularity in peptide synthesis, but now has come evidence that all is not well. It has been reported that phenacyl (Pac) esters of amino acids having bulky side chains are extremely likely to degrade.[40] Further research on the stability of Pac esters is obviously desirable. Two *N*-pentapeptides bearing the –GlySCH_2CH_2COO-Pac moiety at the *C*-terminus were deprotected at both ends of the molecule and formed cyclopentapeptides under catalysis with Ag^+ ions.[41] The separate esterification of the carboxy groups of Asp has been effected by firstly treatment with cyclohexanol in Et_2O in presence of conc H_2SO_4 at 70 °C, acylation with Boc_2O in the presence of Et_3N at O °C and finally esterification of the α-carboxy group with BzlBr in THF in the presence of Et_3N.[42]

New methods of removing protecting groups from blocked carboxy groups have been reported. Methyl esters of Fmoc peptides can be de-esterified using $AlCl_3$/Me_2NPh with preservation of chiral purity.[43] Although the liberation of carboxy groups from Bu^t esters by acids also removes Boc and Trt groups, *N*-9(9-phenylfluorenyl) groups (PhF) survive treatment with $ZnBr_2$ in CH_2Cl_2, conditions that remove other acid-sensitive groups.[44]

2.3 Side-chain protection

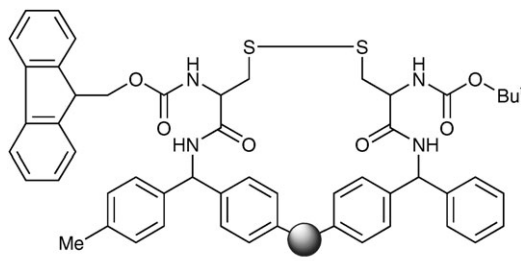
The classical method for protecting the side-chain-amino groups of Orn and Lys involves protection of the α-amino and -carboxy groups as a copper complex. After the introduction of the $N^{\omega(\varepsilon)}$-substituent, the copper can be cleanly removed using $NaBH_4$.[45] The ε-amino group of Lys has been protected with the 9,10-dioxo-9,10-dihydroanthracen-1-yl group and used in SPPS with either Boc or Fmoc chemistry.[46] In order to introduce a substituent on the ε-amino group of one or more Lys residues in a peptide, the peptide is synthesised with Boc blocking the selected amino groups and Dde protecting those amino groups that are required to be in the free state. The Dde groups are then removed with N_2H_4 and replaced with 6-nitroveratryl-oxycarbonyl (Nvoc) groups. The peptide is detached from the resin at this stage and Boc groups are removed and replaced with the desired substituent. Finally, the Nvoc groups are removed photo-chemically by irradiation at 366 nm.[47] This procedure could presumably be used to synthesise a peptide containing both Lys and Arg residues without the need to protect the guanidino group(s). Removal of Troc groups with Zn and Ac_2O also detaches Boc substituents but only the former are sensitive in the presence of Et_3N.[48] This technique is thus an alternative to the foregoing. The guanidino group can be blocked with the CF_3CO– group and deprotected using mild basic conditions.[49] This technique has been used in peptide synthesis. The guanidino group can be protected using the bis(2,2,2-trichloroethyloxy)-phosphinoyl group.[50] Although removal of the ester group was accompanied by formation of mono-2,2-dichloroethyl ester, the subsequent dephosphorylation proceeded satisfactorily.[51]

It has been suggested that if 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT) is used as coupling agent, it is unnecessary to protect the hydroxy groups of Tyr, Ser and Thr and the imidazole group of His.[52] The modest yields of products obtained, however, prevents unreserved recommendation of this technique. Photoirradiation at 245 nm in presence of CBr_4 removes isopropylidene, benzylidene, trityl and Bu^tMe_2Si groups from carbohydrate derivatives such as nucleosides, but the technique does not appear to have been used in peptide synthesis.[53] A gentler method of removing Bu^tMe_2Si groups involves treatment with 30% $KHSO_4$ in MeOH at room temperature.[54] For the protection of the thiol group, treatment with TrtCl in the presence of BF_3 in Et_2O has been recommended and good yields of several small peptides were obtained.[55] 4-Hydroxyphenacyl bromide has also been recommended for protecting thiol groups since deprotection could be effected by irradiation at 312 nm.[56] The formation of thioesters as byproducts in this step suggests that some caution is appropriate. Removal of *S*-Bzl and *O*-Bzl groups can be effected with ammonium formate and zinc dust accompanied by an input of microwave radiation.[57] Many other groups are unaffected by this treatment. The use of N^{im}-2,6-dimethoxybenzoyl histidine (Dmdz-His) has been studied.[58] The site of substitution in the heterocyclic ring did not radically affect either the yield or chiral purity of Fmoc dipeptides. Usually, better results are obtained when the protecting group is sited on the π-N atom. If the Fmoc group is removed while the Dmbz-group is still in place, the latter can be transferred to the α-amino group to some extent. A new method of protecting the amide group with a Trt substituent involves heating a solution of the substrate with TrtOH, CF_3SO_3H under reflux in benzene for 3–15 h. Yields are good to excellent.[59] Although not required for group protection, it is pertinent to mention here that the aromatic ring of Phe can be iodinated using IPy_2-BF_4. The ortho isomer is the major product.[60] Finally, the use of Met(O) in the assembly of the β-amyloid peptide sequence by SPPS interferes with peptide aggregation and thus improves the yield of desired product.[61]

2.4 Disulfide-bond formation

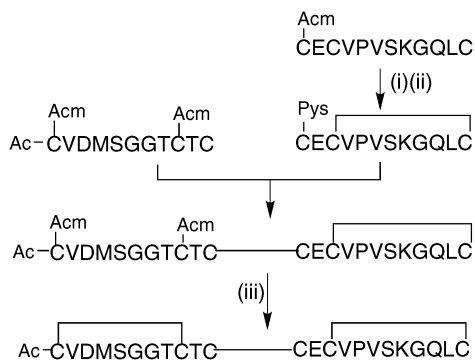
The period since the previous Report has been mainly devoted to consolidation, although a few difficult problems have been solved. Formation of cyclic disulfides

has often started from dithiols that are protected with Acm or Trt groups. Deprotection and oxidation with I_2 under high dilution in order to disfavour intermolecular reaction gives cyclic disulfides.[62,63] Dithiols can be oxidised with H_2O_2 to give disulfides.[64] It is reported that the side chains of Trp, Tyr and Met are unaffected. 4,4′-Dithiopyridine oxidises a pair of thiol groups within a single peptide chain intramolecularly at pH 1.8 to give a cyclic disulfide.[65] Some peptides that consist of alternate L- and D-amino acid residues can switch between linear and cyclic forms if a disulfide group is introduced into the backbone of the molecule. The molecular conformation can be controlled by reduction of the disulfide moiety and reoxidation of the resultant dithiol.[66] It has been shown previously that Ellman's reagent when attached to a PEG-PS support, controlled-pore glass or modified Sephadex oxidises dithiols to disulfides. This technique has been extended to *C*ross-*l*inked *e*thoxylate *a*crylate *r*esin (CLEAR).[67] For example, a urotensin II antagonist peptide containing a hindered penicillamine residue was oxidised satisfactorily. In addition, the two amino groups of immobilised lysine were coupled intramolecularly to the two carboxy groups of Ellman's reagent to give a cyclic disulfide. A mixture of a synthetic tetrapeptide, NH_2CO–Lys–Leu–Gly–Cys–NH_2 (A_{SH}), and a synthetic octapeptide, NH_2CO–Lys–Leu–Lys–Leu–Lys–Leu–Gly–Cys–NH_2 (B_{SH}), were oxidised in air and gave three products, $A_{SS}A$, $A_{SS}B$ and $B_{SS}B$ in the ratio 1.0:2.4:1.1.[68] Similar results were obtained when $K_3Fe(CN)_6$ was used as oxidising agent. Nmr studies revealed that the foregoing disulfides had hairpin structures. A disulfide heterodimer (**5**) has been ingeniously constructed from Boc–Cys(Npys)–OH, Fmoc–Cys(Mmt)–OH and 4-methylbenzhydrylamine resin.[69] A mixture of the two cysteine derivatives (1:1.3) was loaded on the resin using DCC and HOBt. The Mmt group was acid-labile whereas the 2-pyridinesulfinyl group was stable to acid. The Mmt group was removed and the liberated thiol group attacked the Npys group to form a disulfide bridge.



(**5**)

A more complex example of disulfide link formation is shown in Scheme 2.[70] The synthesis of α-conotoxin dimer peptide containing four disulfide links deserves an



Scheme 2 Reagents: (i) MeSOMe (10%) in NH_4HCO_3 aq.; (ii) I_2 then ascorbic acid.

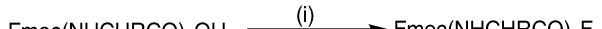
accolade for the most demanding synthetic skill to obtain a product with the desired structure.[71] The synthetic peptide chain had four pairs of thiol groups bearing Trt, Acm, Bu^t and MeBzl that permitted the regioselective formation of the required disulfide links. Trt groups were removed first and the product was oxidised with 33% Me_2SO at room temperature for 24 hr. Next the Acm groups were removed with I_2 leading to the formation of the second disulfide link. Thirdly, the Bu^t groups were removed and the product was oxidised with Me_2SO/CF_3CO_2H. Finally, the MeBzl groups were removed and the product was oxidised with Me_2SO/CF_3CO_2H at 45 °C.

2.5 Peptide bond formation

Success in forming a new peptide bond by coupling two smaller components is measured by the yield of product and by the degree of retention of chiral purity especially in the acylating component of the reaction. For a number of years there have been occasional reports that the presence of copper ions assists in attaining both objectives. It is rather surprising therefore that there are so few publications in which this technique figures prominently. There has been an isolated report[72] that CuCl or $Cu(OBt)_2$ cause good acceleration of the reaction between an *N*-protected peptide thiol ester and a peptide bearing an unprotected amino group.

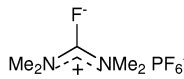
The use of symmetrical or unsymmetrical anhydrides has lost its popularity. Symmetrical anhydrides have often been preferred because nucleophilic attack by the unprotected amino group of a peptide or amino acid derivative can give only one product. The disadvantage of this method is the concomitant release of half of the original anhydride as free acid. Conversely, if an unsymmetrical anhydride (usually incorrectly described as a mixed anhydride) is used, nucleophilic attack can occur on the wrong moiety of the anhydride leading, for example, to a urethane if a chloroformic ester is used to form the unsymmetrical anhydride and the free acid of the component that is required to act as acylating component in the reaction. Moreover, unsymmetrical anhydrides can undergo disproportionation to give one molecule of the two related symmetrical anhydrides with a resultant poor yield of the required product. Victor Gold studied this kind of system extensively in the middle of the last century. There has also been a recent report[73] on this common problem in relation to peptide synthesis. The authors report that changing the experimental protocol by reversing the order of addition of *N*-protected amino acid or peptide to chloroformate decreases the amount of symmetrical anhydride to $<2\%$. Nevertheless, it is probable that the use of symmetrical or unsymmetrical acid anhydrides will not match the performance of other techniques described below despite a report[74] that the use of Fmoc-Cl gave good yields of some dipeptide derivatives with retention of chiral purity. The symmetrical anhydride derived from the treatment of 2-hydroxypyridine *N*-oxide with $(CCl_3)_2O$ could be used as a coupling agent and it did not require the addition of a teriary amine.[75] Chiral purity was preserved in the synthesis of dipeptides but in coupling Z–Gly–Phe–OH and H–Val–OMe, the results were variable.

In contrast, acyl halides, which had been dismissed as effectively useless in view of the extensive loss of chiral integrity, have experienced an increase in use. Fmoc amino acid chlorides have been prepared by the reaction of free acid and $SOCl_2$ with assistance by ultrasound[76] or by the reaction of the free acid with $(Cl_3CO)_2O$.[77] This latter paper describes the synthesis of peptides containing *N*-methylamino acid residues including omphalotin, a cyclic peptide with a particularly challenging structure. *N*-Trifluoroacetyl amino acids (prepared by the reaction of the amino acid with CF_3CO_2Et in MeOH containing 1.0 equivalent of KOMe at 40 °C) were treated with Vilsmeier reagent $(Me_2N^+{=}CHCl)Cl^-$ in BuOAc at −10 °C.[78] The resultant acid chloride was allowed to react with an amino acid under Schotten-Baumann conditions in presence of K_2HPO_4 at 1–4 °C with pH control in the region 7.0–8.0 by addition of NaOH. If the pH is too high, formation of azlactone and loss of chiral purity occur. If the pH is too low, the amino component is largely

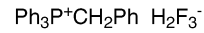


Scheme 3 Reagents: (i) $Ph_3P^+CH_2Ph\ H_2F_3^-$ or $Py(HF)_n$.

protonated. Loss of chiral integrity increases with time so the reaction should be stopped when apparently complete. The use of an autotitrator seems to be indicated in this step. It has been discovered that Fmoc amino acid fluorides are among the most efficient reagents for forming peptide bonds.[79] Although Fmoc amino acid fluorides are less reactive than acyl chlorides, by adding complex polyfluorides to the reaction mixture, the rate of reaction can be enhanced and loss of steric integrity avoided. Other coupling methods using *e.g.* HBTU, HATU or carbodiimides can be diverted to acyl fluoride mechanisms by the addition of complex fluorides (Scheme 3). Other advantages of the use of acyl fluorides include retention of Bu^t and Trt protecting groups and the removal of the need to add tertiary base with the consequent avoidance of the formation of oxazolones. Difficulties were experienced in coupling His in the form of Fmoc-His(Trt)-F because of uncertainty about the long-term stability of the reagent. In addition, Arg derivatives with sulfonamide protection could not be converted into acyl fluorides due to rapid cyclisation to lactams. Some reagents such as (**6**) give unsatisfactory conversion of acids into acyl fluorides. This problem was solved by using (**7**) as an additive.



(**6**)

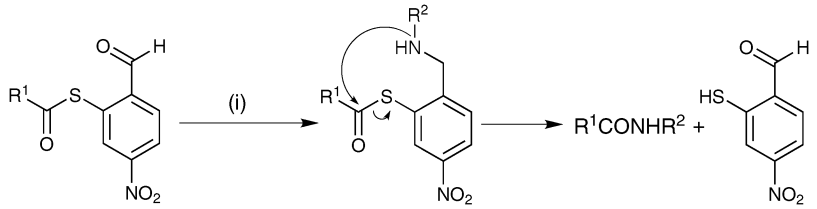


(**7**)

There has been little movement on the carbodiimide front. Following the initial reaction of the carboxy group of the acylating component with the –N═C═N– group to give the *O*-acylisourea, there is a well known tendency for an acyl shift to occur giving the unreactive *N*-acylurea. It has been reported that replacing the *NN′*-dicyclohexylcarbodiimide by *NN′*-diisopropylcarbodiimide avoids this problem.[80] Further, improved yields of product in carbodiimide mediated synthesis can be obtained by addition of pyridinium 4-toluenesulfonate and a tertiary base.[81] It is doubtful, however, if these recommendations will rescue the carbodiimide method from obsolescence. A new coupling reagent, $TaCl_5$, has been recommended because it causes very low loss of chiral integrity and is particularly useful for coupling involving the acylation of *N*-methylamino acid derivatives.[82] For example, coupling of *N*–Z–Ala–OH and *N*–Me–Phe–OEt afforded a yield of 75% with only 0.2% loss of chiral purity. The same reaction when mediated by DCC gave only 50% of product.

A potentially important discovery concerns a method for synthesis in the N → C direction. Although this is the direction of protein synthesis under genetic control, it has traditionally been regarded as unsuitable for chemical synthesis because attempted coupling leads to extensive or even total loss of chiral integrity. An unsymmetrical anhydride of an *N*-protected amino acid, X-NHCHRCOOCOBu^t, is reacted with an *O,N*-bis-trimethylsilylamino acid.[83] Yields are good and chiral integrity is preserved. A helical hexapeptide has been successfuly synthesised and intermediate fragments have been characterised. The recently reported experiment in which amino acids produce peptides in the presence of COS,[84] which could derive from volcanic eruption, suggests that unsymmetrical anhydrides were at work long before nucleic acids came on the scene.

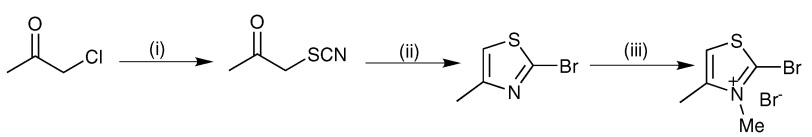
Ionic liquids have not only been used in the esterification of amino acids,[34] but also in coupling reactions involving BOP or HATU.[85] Results with hindered amino acids were encouraging at room temperature. Derivatives of 2-chloro-1,3,5-triazine are well known as coupling agents, so the design of a heavily labelled fluorinated derivative comes as no surprise. 2-Chloro-4,6-bis[(heptadecafluorononyl)oxy]-1,3,5-



Scheme 4 Reagent: (i) R^2NH_2.

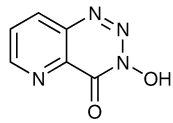
triazine has been used to synthesise small peptides and excess reagent and products thereof are easily separated from product.[86] Fmoc-/Boc-β-amino acids are readily prepared as their diazomethane derivatives by a variation of the Arndt-Eistert reaction and can be used for the preparation of β-peptides.[87] A novel method of peptide synthesis uses 2-formyl-4-nitrophenylthioester as starting material (Scheme 4).[88,89] The reaction is conducted in $CHONMe_2$-phosphate buffer (pH 8.0). The liberated 2-formyl-4-nitrothiophenol is captured by *N*-methylmaleimide *via* 1,4-addition. The method was tested using amino acids with a variety of side chains.

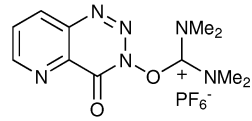
Carpino has continued his search for the ultimate coupling reagent and additive. 3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazene (HODhat, **8**) is a useful additive. Moreover, uronium and phosphonium salts derived from HODhat are useful coupling agents. For example, *O*-(3,4-dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (**9**) was very effective in the synthesis of ACP(**65–74**). HODhat is less suitable in couplings that are effected with DCC due to the formation of byproducts.[90] Further, reaction of HOAt with some phosphonyl and phosphinyl derivatives [$(RO)_2POCl$ and R_2POCl] afforded useful coupling reagents (*e.g.* **10**), that are generally superior to uronium and guanidinium analogues in terms of retention of chiral purity.[91] For example, ACP(**65–74**) was synthesised in 85% yield in a very short time. The phosphinyl reagents have a better shelf stability than the phosphoryl compounds. A new route to 2-halothiazolium coupling reagents has been reported.[92] α-Thiocyanoketones are cyclised with HBr and the resultant thiazole is quaternised by reaction with MeBr (Scheme 5). The method was tested by coupling Boc–MeIle–OH and MeIle–OBzl. This was a searching test and a good yield was obtained with no detectable loss of chiral purity. As a result, it was suggested that it was unnecessary to add HOBt during the coupling step. Carpino has reported a difficulty during the synthesis of peptides of Ser and/or Thr.[93] LC-MS had revealed the presence of impurities with MW identical to the desired product. One of the impurities resulted from loss of chiral integrity involving Ser. This problem was largely solved by decreasing the preactivation time before coupling. Other peaks of identical MW were attributed to $N \rightarrow O$ acyl shift during the acidic removal of protecting groups. Nevertheless, some syntheses were classified as difficult. The ingenious solution devised included deliberately involving the tendency of acyl groups to migrate. Fmoc group was detached and replaced by Boc. Next, OTrt groups were removed from Ser/Thr side chains using 1% CF_3CO_2H in CH_2Cl_2 in presence of Et_3N. The next residue was introduced on the hydroxy group using Bsmoc-amino acyl-fluoride in the presence of DMAP to give a depsipeptide (Bsmoc = 1,1-dioxobenzo[b]thiophen-

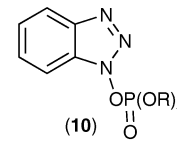


Scheme 5 Reagents: (i) NaSCN, EtOH; (ii) HBr; (iii) MeBr.

2-ylmethoxycarbonyl). The synthesis was continued after removal of the Bsmoc group and no problems were encountered. At the end of the synthesis, the *O*-peptidyl group was caused to migrate to the amino group using aqueous ammonia.

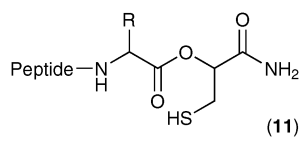

(**8**)

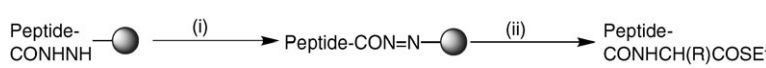

(**9**)


(**10**)

The Staudinger ligation method was described in the last Specialist Report[1] and has since been studied more thoroughly.[94] Tetra- and penta-peptides have been synthesised starting from α-azidopeptides as *N*-terminal components and *O*-(diphenyl-phosphino)phenyl esters as *C*-terminal fragments. In what can only be described as a great leap forward so soon after the invention of the method, ribonuclease A has been synthesised.[95] The enzyme molecule was assembled from three fragments, **1–109**, **110–111** and **112–124**.

In the further development of the native chemical ligation (NCL) technique reported in the previous volume of this series, considerable effort has been deployed to produce peptide thioesters that are required as one ingredient required in the ligation method. *N*-Acylbenzotriazoles react with a variety of thiols giving rise to thioesters in good yield.[96] It can be noted here that *N*-acylbenzotriazoles react with amino acids or peptides to give *C*-terminally extended peptides.[97] Peptides having a free carboxy group at the *C*-terminus can be converted into thioesters by reaction with a thiol in the presence of PyBOP and Pr^i_2NEt. The preferred thiol is 4-acetamidothiophenol because it reacts faster than with $HS(CH_2)_2COOEt$.[98] This procedure was carried out with pro-NPY(**1–40**) and protecting groups were removed with CF_3CO_2H before ligation with pro-NPY(**42–69**) thus giving an analogue of native NPY. The preparation of thioesters is complicated by their sensitivity to aminolysis by the secondary amines used to remove the Fmoc group. The problem can be overcome by masking the thioester as a trithioortho ester.[99] The protecting group is retained during the synthesis. An alternative approach uses Boc chemistry so that exposure to bases is avoided.[100] A further alternative route to peptide thioesters involves assembling the *N*-terminal fragment of the ultimate product by SPPS on a support bearing aryl-hydrazine groups and the *C*-terminal thioester group is not introduced until all the amino acid residues have been incorporated.[101] Oxidation then of the hydrazide moiety with *N*-bromosuccinimide gives rise to an acyl diazene which can undergo aminolysis by treatment with an amino acid thioester (Scheme 6). The procedure has been used both in NCL of peptides and in cyclisation of peptides. Yet another approach to the synthesis of peptide thioesters required for NCL involves the preparation of an oxy-(2-mercapto-1-carboxy-amido)ethyl ester (**11**).[102] The latter undergoes $O \rightarrow S$ acyl shift during NCL and the resultant thioester reacts with the *N*-terminal Cys residue of the intended *C*-terminal fragment.


(**11**)

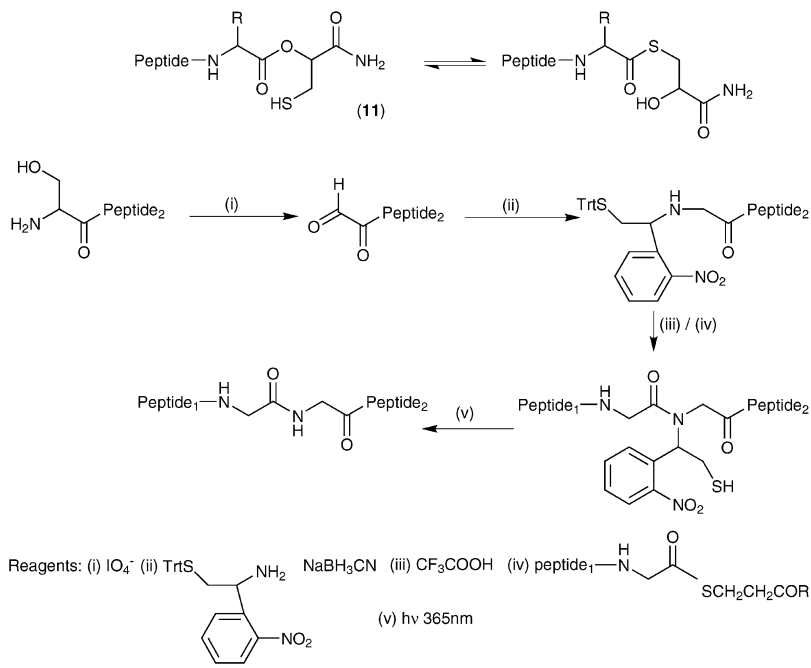


Scheme 6 Reagents: (i) N-Bromosuccinimide; (ii) $NH_2CH(R)COSEt$.

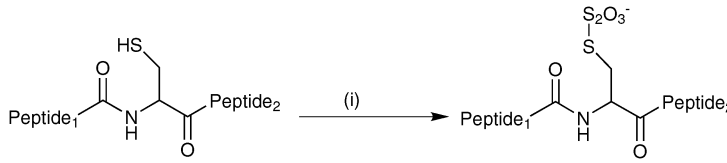
Problems have been encountered when the intended *N*-terminal fragment has either Glu or Asp as the *C*-terminal residue.[103] If the β-group of Asp or the γ-group of Glu was left unprotected, the product from NCL with a peptide having an *N*-terminal Cys residue consisted of two substances of identical molecular weight. In each case, one product was resistant to hydrolysis by V8-proteinase. Similarly, in the case of Glu, the resistant product was clearly the γ-peptide. The problem was solved by protecting the relevant carboxy group. Phenylsulfonylethyl ester was best for Glu and 1-methyl-2-oxo-phenylethyl ester was preferred for Asp.

A photosensitive auxiliary group, 2-mercapto-1-(2-nitro-phenyl)ethylamino, has been used to generate a Gly residue at the site of of the new linkage generated in NCL.[104,105] A peptide with an *N*-terminal Ser residue replaces Cys at the *N*-terminus of the *C*-terminal fragment. It is oxidised with periodate and then subjected to reductive amination with the auxiliary reagent. The latter is detached photochemically at 365 nm (Scheme 7). This appears attractive because a Gly residue commonly occurs in peptides so it would offer a more catholic choice of ligating sites. The same result can be achieved using 4,5,6-trimethoxy-2-mercaptobenzyl- and 1-(2,4-dimethoxyphenyl)-2-mercaptobenzyl-auxiliary groups.[106] These groups can be detached with CF_3CO_2H. A similar method that required more vigorous conditions to detach the auxiliary group was mentioned in the previous Specialist Report.

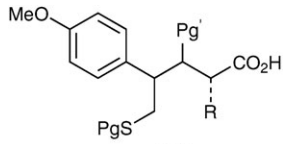
If it is desired to attach another peptide to the product of NCL, there will be a free thiol group on the product of the first reaction. If desired this can be protected temporarily as a thiosulfonate group by mixing with sodium tetrathionate (Scheme 8).[107] Deprotection can be effected with dithiothreitol. Compounds of type (**12**) have been synthesised with the intention of using them in NCL.[108,109] Derivatives of ε-cysteinyl lysine (**13**) have been synthesised and used in NCL.[110] It was postulated that the availability of orthogonally protected amino groups and a carboxy group invited the synthesis of a scaffold for NCL.



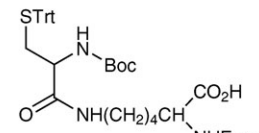
Scheme 7



Scheme 8 Reagents: (i) $Na_2S_4O_6$.



(12)



(13)

The number of peptides that have been obtained by the NCL methodology is legion and only a few will be mentioned here. A semi-synthetic proteolytic enzyme derived from *S. griseus* was prepared by NCL using a chemically synthesised fragment and a peptide obtained from a genetically engineered protein obtained by chemical cleavage with CNBr.[111] The product displayed kinetic properties identical to those of the native enzyme. One of the earlier albeit smaller successes was the synthesis of crambin, a protein containing 46 residues from the seeds of the plant, *Crambe abyssinica*.[112,113] Although this was synthesised twice from two components (31 + 15 and 15 + 31), best yields were obtained from three fragments containing, respectively 15, 16 and 15 residues. This suggests that although the use of large fragments may be spectacular, components of a more modest size may sometimes provide a better opportunity to obtain a product with the correct molecular conformation (see Section 2.8). Finally, a synthetic erythropoiesis protein consisting of a polypeptide chain of 166 residues linked covalently to two monodisperse polymer moieties was made by NCL and had potent biological activity with a prolonged duration of biological activity *in vivo*.[114] It is to be hoped that a similar product never becomes available on the athletics black market.

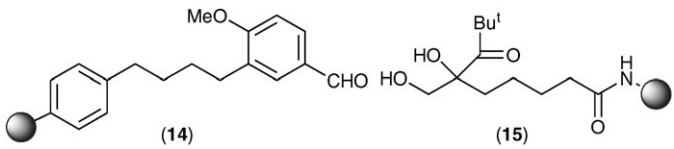
2.6 Peptide synthesis on macromolecular supports

Some desirable characteristics of solid supports for SPPS are mutually incompatible. For example, the higher the capacity for attaching peptide, the greater the possibility of interaction or steric hindrance occurring between peptide chains and the structural elements of the support. Hence, it is likely that poor coupling yields would occur as the peptide chains grow. Again, the greater the hydrophobicity of the support, the greater the chance of noncovalent interactions occurring that involve the side chains of Trp, Phe, Leu, Ile and Val. It is not surprising, therefore, that new supports are constantly being sought. There has been a tendency to prepare new supports that are partially hydrophilic and have their chains well separated so that the support swells during coupling stages. The incorporation of PEG, for example, contributes no sites to attach peptides but helps to separate polymer chains and is hydrophilic. Derivatives of glycols and other polyhydroxyl compounds have commonly been incorporated into SPPS supports in order to make them less hydrophobic.[115–120] Although most supports are made from two components, occasionally the composition of the polymer is more complex. For example, one support[120] was constructed from 1-[2-(2-methoxyethoxy)ethoxy]-4-vinylbenzene, styrene, 4-vinylbenzyl bromide and divinylbenzene, and had a loading capacity of up to 1 mmol/g. The resin had good swelling properties and a test synthesis of a tripeptide gave a good yield of pure product. There remains a concern, however, about the

reproducibilty of structure and properties of different batches of a polymer made from four components. A polymer has been prepared from a partly methacrylated di-*O*-isopropylidene sucrose.[121] The isopropylidene groups were removed and SPPS of a 19-mer peptide from foot-and-mouth disease virus was carried out after incorporation of a linker on the support. Supports have been made from polyethyleneimine crosslinked with terephthalaldehyde and fitted with various linkers.[122] This polymer had a high capacity and swelled in both polar and nonpolar solvents.

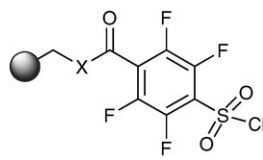
The desired characteristics of a satisfactory linker are few in number but not easy to achieve. During the process of chain elongation, the linker should keep the construction site free of the scaffolding provided by the polymeric support and should not interfere with the chemistry involved in the synthesis. When the latter is complete, the attachment to the linker must be cleaved by the mildest possible method. The days of treatment with HF are long past.

Two linkers derived from 4-methoxybenzaldehyde (*e.g.* **14**) have been designed and are stable during cleavage of Fmoc and Boc groups.[123] The resultant peptides are detached in refluxing CF_3CO_2H. A photolabile linker (**15**) offers the opportunity to remove the *N*-protecting group after each coupling stage at 360 nm and to detach the peptide finally at 305 nm.[124]



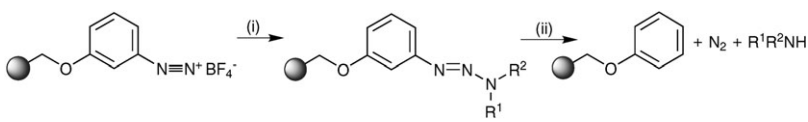
(**14**) (**15**)

Three types of cleavage from the support have been defined;[125]—(*a*) cyclative cleavage whereby intramolecular cyclisation effects peptide detachment, (*b*) traceless cleavage in which the point of attachment is replaced by a C–H bond and (*c*) multifunctional linkers which can yield different products depending on cleavge conditions. A small linker (**16**) was designed that was suitable for traceless cleavage and Pd(0) catalysed cross-coupling reactions. Attachment of peptide can be accomplished *via* a Tyr hydroxy group.



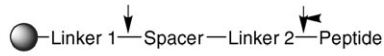
(**16**) (X = O, NH)

Linkage to the support before SPPS can be effected *via* a hydrazide group[101,126] that can subsequently be oxidised to cleave the peptide from the support. Met is reported not to be affected by the oxidation step. It is also possible to link a benzenediazonium salt that is attached to the insoluble support to an amine or amino acid giving a triazene (Scheme 9).[127] The amine or amino acid is cleaved with a laser providing a beam of 355 nm wavelength. This resin and linker provides an opportunity to carry out SPPS in the $N \rightarrow C$ direction although none seems to have been reported (see Section 2.8). Guanidine-bridged linkers have been prepared and used for both solution- and solid-phase synthesis.[128] Ligands against cholera toxin



Scheme 9 Reagents: (i) R^1R^2NH; (ii) hν 355 nm$_2$.

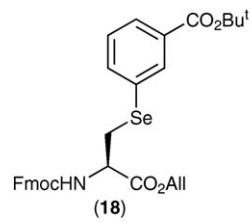
were synthesised. A (2-phenyl-2-trimethyl-silyl)ethyl linker that is sensitive to fluoride ions has been designed.[129] Detachment of peptide is achieved under almost neutral conditions using $Bu_4NF \cdot 3H_2O$ in CH_2Cl_2. Avoidance of acidic hydrolysis permitted the synthesis of glycopeptides. Another advance in linker design involves the use of dual linkers (**17**).[130] The idea behind the design of this type of linker is to ensure that at least one of the two cleavable bonds would be broken and the synthetic peptide would be detached from the resin.



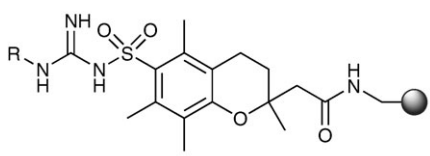
(**17**)

α-Fmoc-Ser(or Thr)-OAll have been converted into (**18**) by reaction with 3-(t-butyloxy carbonyl)selenocyanate and Bu_3P; the product can be coupled to an amino resin. The molecule can be extended at either or both ends then treatment with H_2O_2 in tetrahydrofuran eliminates the Se leaving an dehydropeptide.[131]

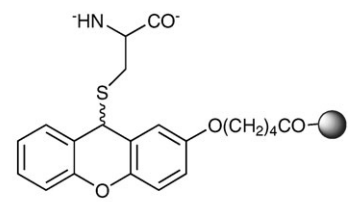
The ω-amino group of α-Fmoc-Orn-OR has been converted into an isothiocyanate group and coupled to the amino group of a Rink linker which is attached to a support.[132] The resultant thiourea is then methylated with MeI producing a guanidino group. After construction of the required peptide chain, the Arg-peptide is detached from the resin using CF_3CO_2H. Presumably, the same approach could be made towards the synthesis of peptide of homoarginine starting out from an ester of α-Fmoc-Lys. An alternative approach starts directly from a derivative of Arg.[133] The linker is derived from (3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)acetic acid which is coupled to an amino resin and the linker is chlorosulfonated with $ClSO_3H$ in $CHCl_3$ in three stages. The linker is now an insolubilised Pmc reagent (**19**). It is coupled to the guanidino group of Arg and can be used as the starting material for SPPS. The product is detached from the resin with CF_3CO_2H. The last method of linking the side chain of an amino acid to the resin for SPPS involves attachment of a *S*-(5-carboxy-n-pentyloxy)xanthenyl linker to the resin (**20**). The peptide can be constructed on the amino and/or carboxy group of the cysteine moiety.[134] Detachment of the peptide can be effected by treatment with 5% CF_3CO_2H. If two cysteinyl groups are present at an appropriate distance, inclusion of DMSO in the cleavage cocktail causes intramolecular oxidation to the disulfide. Alternatively, the oxidation step can be delayed until the peptide has been released from the resin.



(**18**)



(**19**)



(**20**)

Apart from linker moieties, two studies with protecting groups must be mentioned. Loss of peptide loading when removing Boc groups using the Wang resin can

be undesirably high but acidic conditions can be avoided by using trimethylsilyltriflate as deprotecting agent.[135] Although this is not satisfactory for repetitive cycles, it is very suitable for a particular stage in a complex synthesis. In order to be able to carry out SPPS in aqueous solution, the *N*-2-[phenyl(methyl)sulfonio]ethoxycarbonyl group has been recommended.[136] It is introduced by the stable reagent 2-[phenyl(methyl)sulfonio]ethyl-4-nitrophenyl carbonate. This does not affect Met or Cys.

Some special coupling reagents for SPPS have been described. Uronium derivatives of polymeric *N*-hydroxysuccinimide have been synthesised.[137] The polymer liberated after coupling can be recovered and recycled. A more ambitious exercise resulted in the successful synthesis of a 95-mer peptide using the dimethyl-oxazolidine dipeptides of Ser and Thr.[138] The presence of the heterocycle as described earlier by Mutter prevents association reactions that had resulted in a failed attempt to synthesise the peptide from amino-acid building blocks. Regeneration of the Ser/Thr residues occurred during cleavage from the resin with 95% CF_3CO_2H. Mutter's method is recommended as the best technique for avoiding aggregation problems during SPPS.

The formation of aspartimide and related byproducts during SPPS continues to be troublesome. The synthesis of a series of hexapeptides based on the sequence H–Val–Lys–Asp–Xaa–Tyr–Ile–OH has been studied in detail.[139,140] Although several β-ester groups offered an improvement, complete avoidance of this side-reaction required the use of the Bu^t ester and the Hmb backbone protection if Xaa was a Gly residue. When Xaa consisted of Asp or Cys derivatives or unprotected Thr, considerable amounts of byproduct were formed. Milder methods for the detachment of Fmoc groups improved the situation, a result supported by the work of another group.[141]

The recent interest in fluorous chemistry in SPPS may afford a new dimension to peptide chemistry or it may disappear without trace like the once popular tetraethyl pyrophosphite coupling reagent. One group has experimented with various fluorous supports, but has reported the synthesis of only small peptides.[142,143] On the other hand, the use of $C_7F_{15}I^+Ph\ X^-$ as a capping reagent to prevent further coupling involving incomplete chains seems a very useful technique.[144] The products from tagging of free amino groups do not react with coupling reagents and are stable to neat CF_3CO_2H and anhydrous HF.

Although the collection of rate data for SPPS is less exciting than constructing a large peptide with an expected biological activity, the two topics are connected. The kinetics of coupling of the Rink linker with the copolymer of 1,4-butane-diol dimethacrylate have been determined as have the stepwise assembly of a 14-mer peptide on the support.[145] Several hours might be saved in the synthesis of a much larger peptide if the kinetics for each stage are able to be estimated with reasonable accuracy. Part of the time involved, albeit a small fraction of the whole, might be saved if the diffusion coefficients of the reactants into the resin are known. The diffusion coefficients of several *N*-protected amino acids in the presence of various resins have been measured[146] and it is to hoped that more of this work will be undertaken.

Two peptides that have apparently displaced ACP(**65–74**) from its status as the sequence offering the greatest difficulty in synthesis are humanin and **β**-amyloid (**1–40**). The former contains 24 amino acids with the sequence:- MAPRGFSCLL LLTSEIDLPVKRRA and is of importance because it antagonises the neurotoxicity associated with Alzheimer's disease. It has been synthesised manually on a resin of 2-Cl-Trt-aminomethyl-polystyrene using Fmoc chemistry.[147] Couplings were effected with diisopropyl-carbodiimide/HOBt and several stages had to be repeated. In addition, deprotection steps had to be prolonged. Capping techniques were also applied. Since Cys occurs within the most difficult region, it is possible that NCL would have been a better choice of technique, since hydrophobic protecting groups on Ser and Thr would have been unnecessary. In the synthesis of **β**-amyloid(**1–40**), it

is thought that Met^{35} is responsible for promoting oligomerisation. Consequently, Met(O) replaced the unsubstituted amino acid and at the end of the assembly process, the product was reduced with $SiCl_4$/anisole/CF_3CO_2H (5:5:90).[148] Coupling was effected with HBTU.

Finally, although peptide synthesis in the same direction as in the cell ($N \rightarrow C$) has been effectively abandoned, it may yet be exhumed (see Section 2.8). Consequently, it is interesting that a method of SPPS in that direction has been described.[149] Polystyrene bearing some hydroxymethyl groups is treated with $COCl_2$ and the product is used to acylate the Bu^t ester of the *N*-terminal amino acid of the peptide to be synthesised. Hydrolysis is achieved using CF_3CO_2H/CH_2Cl_2. The second amino acid is coupled using HATU. Loss of chiral purity is $<2\%$ which is still unacceptably high for a peptide of any significant length. The reason for wanting to synthesise peptides in the $N \rightarrow C$ direction is to try to ensure that the product has the conformation of the natural peptide.

2.7 Enzyme-mediated synthesis and semisynthesis

Much of the research on enzyme-catalysed peptide synthesis that has been published during the last two years is either a confirmation or a modest extension of earlier work. For example, heptyl esters of *N*-acylamino acids are hydrolysed by lipases.[150,151] Also esters of *N*-acetyl-tyrosine undergo transesterification in the presence of chymotrypsin in MeCN.[152] The velocity of reaction and yield are sensitive to the environment including the solvent; immobilisation of the enzyme on an insoluble support often favours synthesis.[153–157] An explanation of the latter point has been advanced.[156] It is suggested that the microenvironment within the $PEGA_{1900}$, for example, is more hydrophobic than that of the bulk aqueous solution. It would be expected that a substrate bearing a bulky Fmoc group would be transferred from the aqueous solution to the insoluble support. Results with amino acids bearing a bulky hydrophobic group would be expected to give better yields than more hydrophilic substrates. Thus Fmoc-Phe-Phe-support was formed in 99% yield whereas Fmoc-Ser-Phe-support was obtained in only 10% yield; thermolysin was the catalyst. There is not a complete correlation between yield and log P (to quantify hydrophobicity), however, since Fmoc-Gln-OH, which has a very low value of log P, gave a good yield of dipeptide derivative. Further study seems to be required. The use of neat organic solvents frequently favours good yields, especially with immobilised enzymes.[152,158–161]

A number of publications describe the synthesis of small peptides[158,159,162–175] and this adds to the vast amount of information produced during recent years. Nevertheless, there are many variables such as choice of enzyme, the physical state of enzyme (*e.g.* immobilised, dissolved, component of a 2-phase system), nature of substrates such as choice of ester type in the acylating component, nature of medium and temperature. Consequently, extensive experimental variation may be required to optimise conditions. Although these problems may deter some workers from using enzymatic methods of peptide synthesis, there remain some distinct advantages such as maintenance of chiral purity (but see below) and the availability of techniques for ensuring that hydrolysis of substrates or products does not interfere.

Several types of unnatural substrates have been used. Peptides containing D-amino acids are accessible using a 4-guanidinophenyl ester as substrate in the presence of clostripain.[176] Similar esters of α-fluoroalkylacids can be coupled using trypsin in frozen solution.[177] As expected, thioesters are good substrates for the enzymatic acylation of amino acid esters. Starting from a methyl thioester of a protected amino acid, 4-guanidinophenyl, benzyl and 2-carboxy-ethyl thioesters can be prepared by chemical transesterification and the products are substrates, respectively for trypsin, chymotrypsin and V8 proteinase.[178] This approach could form the basis of a SPPS method of enzymatic production of peptides.

Two publications describe ambitious enzymatic syntheses. One describes the SPPS of a linear decapeptide precursor of gramicidin S and its cyclisation on resin using gramicidin thioesterase.[179] The methodology enables a study to be undertaken of the relationship between structure and antibiotic activity. The other study involved the removal of an octapeptide from insulin using trypsin to cleave an arginyl bond.[180] The larger resulting fragment was then coupled enzymatically to *e.g.* opioid peptides using trypsin in a partially nonaqueous medium.

2.8 Epilogue

Since this is the last Specialist Report on peptide synthesis and since the present Reporter has been looking backwards for the last 16 volumes of this series, it is perhaps appropriate to look forward briefly. In 1987, a view was expressed by a molecular biologist that with the arrival of automatic peptide synthesisers, there surely was nothing more to investigate. After writing nearly 900 pages and citing over 10 000 references, a case of chemical and biochemical myopia in the molecular biologist is suspected.

It would be wrong to classify the most important advances as milestones. The latter are rapidly passed by a motorist or may not even be seen. There have been a number of developments that have changed the field of peptide synthesis that cannot be forgotten. We have seen new coupling reagents and systems that have led to improved yields of products with miniscule loss of chiral integrity. Solid phase techniques have made it possible to synthesise long peptides. It is only about 50 years since Vincent du Vigneaud was excitedly reporting the synthesis of oxytocin and vassopressin. Numerous protecting groups are now available, some of which are detachable under extremely mild conditions including enzymic techniques. The recent discovery of native chemical ligation has not only facilitated the synthesis of long peptides but also has almost eliminated the need for protecting groups on the side chains of amino-acid residues. What else needs to be done? There is the nagging worry that chemists synthesise peptide chains in the opposite direction from that used in the cell; do the natural and synthetic products have the same conformations? In the present review, two papers report peptide synthesis in the $N \rightarrow C$ direction, but it is unlikely that many researchers will change the habits of a lifetime. Nevertheless, it is desirable that some structural studies by NMR spectroscopy and X-ray crystallography be carried out to compare the conformations of peptides that are synthesised in opposite directions. It would perhaps be best to look at proteins that do not contain disulfide bonds which would almost certainly influence molecular conformation.

The usual way to carry out NCL is to join two peptides with the peptide that will be nearer to the *C*-terminus having a cysteine residue at its *N*-terminus. The view has been expressed that Cys is rather uncommon hence requiring the ligation of rather large fragments. Here is a suggestion for future work. A thiol group in the side chain of Cys will react with ethyleneimine or a protected 2-aminoethyl iodide to give either immediately or ultimately *S*-(2-aminoethyl)cysteine or thialysine. The latter must resemble lysine very closely because á-toluene-4-sulphonyl-L-thialysine methyl ester hydrochloride is a good substrate for trypsin as was shown by this Reporter and colleagues almost four decades ago. A good further test of this thesis would be to synthesise a mutant of RNase in which Lys^{7} and Lys^{41} are replaced by thialysine. This would entail using NCL to link fragments (**1–6**), (**7–40**) and (**41–124**). The enzymatic activity of the mutant enzyme could be compared with that of the native enzyme. Moreover, the structures could be compared by X-ray diffraction studies.

There is still some reluctance on the part of some chemists to use enzymes as synthetic tools. In the case of proteolytic enzymes, those involved in protein degradation have evolved so that a very small number cope with a plethora of food proteins and so are not particularly specific. With the advent of NCL, however, the opportunity exists for some protein engineering in the substrate-binding site to

enhance specificity. The constraints of genetic engineering to use coded amino acids are no longer relevant. The advent of synthetic D-peptidases would facilitate the removal of any traces of products produced by loss of chiral purity. To conclude, the words of Lord Tennyson seem pertinent:-

"All experience is an arch wherethro
Gleams that untravelled world, whose margin fades
For ever and for ever when I move."

3. Appendix: A list of syntheses in 2003–2004

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.

3.1 Natural peptides, proteins and partial sequences.

Acyl carrier protein		
	Retro ACP(**65–74**)	181
Adaptor protein		
	Grb2 antagonists	182
	Peptides binding to SH2 domain	183
	Grb2-SH2 domain antagonists	184
Amyloid peptides		
	Amyloid fragments of β2-microglobulin	185
	Isopeptide of Aβ1–42 found in Alzheimer's disease	186
Angiogenesis		
	Anginex, an antiangiogenetic peptide	187
Angiotensin		
	Analogues	188–192
Antibiotics		
	E. coli R1 plasmid host killing peptide	193
	Efrapeptin	194
	Gramicidin S and analogues	179,195–200
	Indolicidin and analogues	201,202
	Trichovirin	203
	Tentoxin and analogues	204
	Capreomycin IB	205
	Globomycin analogues	206
	Polymyxin B1 and analogues	207,208
	Mannopeptimycin analogues	209
	Zervamycin II-2 (**6–16**)	210
	Bleomycin	211
	Peptides from *C*-terminus of a haemolytic lectin	212,213
	Amythiamycin D	214
	4-Fluorinated UDP-MurNAc pentapeptide	215
	Bactenecin 7	216
	Maculatin 1.1 from tree frog (*Litoria genimaculata*)	217
	Cyclosporin A (tritium labelled)	218

	Peptides that bind to bacterial lipopolysaccharide	219
	Nocathiacin I analogues	220
	Alamethicin analogues	221
Aquatic peptides		
	Aeruginosin E1461	222
Bombesin		
	Analogues labelled with ^{99m}Tc	223
Bradykinin		
	Analogues	224,225
Calcitonin		
	Tc-99m-labelled calcitonin	226
Calcitonin gene-related peptide		
	Analogues	227
Chemotactic peptides		
	Analogues	228–234
Cholecystokinin and gastrin		
	Canine CCK-58	235
	NCL synthesis	236
	Enzymatic synthesis of Phac–Met–Gly–Trp–Met–Oet	237
Collagen		
	Fragment analogues	238–242
Corticotropin-releasing factor (CRF)		
	CRF antagonists	243,244
	Analogue and fragments of locust peptide	245
Defensins		
	Human defensins 4, 5 and 6	246
Elastin		
	Fragment analogues	247,248
	Analogues	249
Erythropoiesis protein		
	NCL of analogue	114
Fungal peptides		
	Fragment analogues of fungal kinesin	250
Glutathione		
	Analogues	251–253
GNrh/LHRH		
	Analogues	254,255
Ghrelin		
	Rat ghrelin	256
Growth-hormone releasing factor (GHRH)		
	Analogues	257–262
Hemiasterlin		
	Analogues	263–265
Histogranin		
	Analogues of fragment **7–10**	266
Hypocholesterolaemic peptide from *Soja hispida* seed		
	Fragments	267

	Aah I toxin of *Androctonus australis hector*,	381
	Pardaxin, shark repellent toxin	382
Transthyretin		
	Analogues with enhanced amyloidogenic properties	383
Urotensin		
	Analogues	384,385
	Urotensin II related peptide	386
Viral proteins		
	Fragment of hepatitis G virus	387
Vasoactive intestinal peptide (VIP)		
	Receptor binding inhibitor	388
Zn finger peptides		
	Peptides containing CPLC at molecular terminus	389
	Peptides containing trispyridine-Ru or -Os	390
	NCL synthesis of DNA binding domain of Sp1	391,392

3.2. Sequential oligo- and poly-peptides	
Silk model polypeptides $[Ala–Gly]_{12}$	393
Stereocontrolled polymerisation of amino acid NCAs	394
Polyaspartic acid	395
$[Ala–Ala–Pro]_n$ n = 2,3,4	396
$[Xaa–Pro–Pro–Pro]_n$ n = 2, 3, 4; Xaa = Ala, Leu, Val	397
Xaa_n; Xaa = chiral α,α-disubstituted α-amino acid	398
Peptoids containing *N*-heterocycles	399
Polymers of β-amino acids	400
$[Xaa–Xaa–Xaa–Pro]_n$ Xaa = Glu, Asp, Lys	401
$[Xaa–Xaa–Pro]_n$ Xaa = Glu, Asp, Lys, Leu, Val	402
$[D\text{-}Xaa\text{-}Pro]_n$ Xaa = Ala, Asp, Glu, Lys	403
N-Blocked $[Tyr(sulphate)]_n$	404
SPPS of oligopeptides using pentacoordinated phosphoranes	405
Poly-Lys crosslinked by KGYG tetrapeptide units	406
AAB- and ABC-type heterotrimeric α-helical coiled coils	407
Conjugates of poly-Lys with porphyrins	408
3.3. Enzyme substrates and inhibitors	
Inhibitor of cathepsin L	409
Inhibitors of dipeptidyl dipeptidases	410–415
Inhibitors of papain	416
Peptide vinyl sulfones and cysteine proteinases	417,418
Calpain inhibitors	419–425
Inhibitors of malarial aspartyl proteinases	426,427
Kinetics of γ-glutamyl transpeptidase	428
Synthesis of (-)-tetrahydrolipstatin	429
Inhibitors of cathepsin C	430
Paecilopeptin, a cathepsin S inhibitor	431
Inhibitors of propyl hydroxylase	432
Inhibitors of neuronal nitric oxide synthase	433

3.5. Glycopeptides

3.9. Miscellaneous peptides

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Analogue and conformational studies on peptides, hormones and other biologically active peptides

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1. Introduction

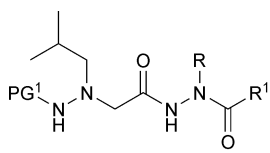
The sub-divisions and the basic structure of this Chapter remained unchanged compared to previous volumes. Core references for this Chapter were obtained from the Chemical Abstract Service (CA Selects on Amino Acids, Peptides and Proteins). The expansion in the availability of scientific journals in electronic format and computer scanning of published titles has become much easier and gave us a big help in composing the material of this Chapter. The following Web of Science databases were used: http://www.ncbi.nml.nih.gov/entrez/query.fcgi, http://isinet.com/isi/products/citation/wos. However, as in the past, conference reports are not reviewed in this Periodical Report. No patents have been used as source material.

2. Peptide backbone modifications and peptide mimetics

A 212-reference review discussed the lack of general success with retro-inverso peptide isomers.[1] Recent finding on immunological applications as well as progress towards generically applicable synthetic methods were also summarized. A series of different peptidomimetic agonists for the human orphan receptor BRS-3 containing azaglycine, piperazine, piperidine, semicarbazide or semicarbazone building blocks has been reviewed.[2]

2.1 Aza, oxazole, thiazole, triazole and tetrazole peptides

Hydrazino-azapeptoids having the general formula of (**1**) with therapeutic potential as anticancer agents were designed and synthesized.[3] Azapeptide epoxides containing an azaasparagine residue proved to be selective inhibitors of *Schistosoma mansoni* and pig kidney legumains, which are clan CA cysteine proteases.[4] To investigate solvent effect on conformational stability, the polarizable continuum model (PCM) and the isodensity polarizable continuum model (IPCM) were applied for azaglycine-containing dipeptides, Ac–Ala–azaGly–NH_2 (**2**) and Ac–Phe–azaGly–NH_2 (**3**).[5] The conformational influence of azaproline in stabilizing reverse-turn conformations in peptides was determined both computationally and experimentally by the synthesis and NMR investigations of [azaPro^3]-TRH and [Phe^2, azaPro^3]-TRH.[6] The natural bond orbital (NBO) analysis was performed to understand the origin of the rotational barrier for the N–N bond in azapeptide using a model compound, *N*,*N*′-diformylhydrazine.[7]



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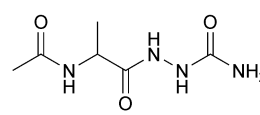

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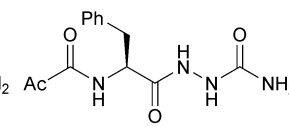

3

Total synthesis of dendroamide A (**4**), a multidrug-resistance reversing peptide-derived oxazole- and thiazole-containing macrocycle has been reported.[8] A new family

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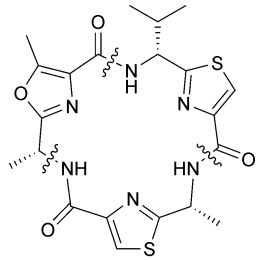
of densely functionalized oxazole-containing amino acids having the general formula of (**5**) was prepared.[9] These building blocks were used for the synthesis of macrocycles as orthogonally protected scaffolds for supramolecular chemistry. A combinatorial library based on 1,3-azole containing peptides (**6–9**) was prepared and screened for activity against matrix metalloproteinase-14.[10] An easy and new method was developed for synthesizing 3,4,5-trisubstituted 1,2,4-triazoles (**10**) that can be applied to acids and amines including α-amino acids.[11] Synthesis and inhibitory potencies of three types of protease inhibitors of the hepatitis C virus full-length NS3 (protease–helicase/NTPase) have been reported.[12] Bioisosteric replacement of the carboxylate with the tetrazole group provided inhibitors equally potent to the parent compounds.



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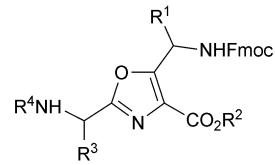

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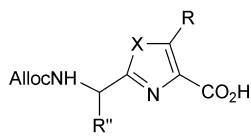
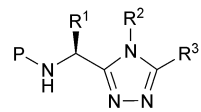


6 X = O, R = CH_3, R'' = H
7 X = O, R = *n*Bu, R'' = H
8 X = O, R = CH_3, R'' = CH_2STrt
9 X = S, R = CH_3, R'' = H

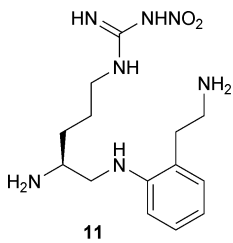


10

2.2 Ψ[CH_2NH], Ψ[CH═CH], Ψ[CF═CH], Ψ[CH(OH)–CH_2], Ψ[CO–CH_2], Ψ[CH_2CH_2NH], Ψ[CH(OH)–CH_2–NH], retro- and retro-inverso-Ψ[NHCH(CF_3)], retro-Ψ[$NHCH_2$], retro-inverso-Ψ[CONH], Ψ[CO–NH–O], Ψ[CH═N–O], Ψ[CH_2–NH–O], Ψ[CH(OH)–CO–N(R)–NH], Ψ[NH–CO–NH], Ψ[N═S(R)(O)], Ψ[PO(OH)–CH_2]

A series of iso-lactam and reduced amide analogues of the highly potent peptidomimetic dopamine receptor modulator 3(*R*)-[(2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide have been synthesized and tested for their ability to enhance the binding of $>{}^3$H]*N*-propylnorapomorphine to dopamine receptors in a functional *in vitro* assay.[13] To increase the lipophilicity of previously reported neuronal nitric oxide synthase (*n*NOS) inhibitors, a series of aromatic, reduced amide bond analogues were designed and synthesized.[14] The most potent compound among them, *N*-(4*S*)-{4-amino-5[2-(2-aminoethyl)phenylamino]-pentyl}-*N*′-nitroguanidine (**11**) (K_i = 50 nM) showed greater than 2100-fold selectivity over endothelial NOS. Two Merozoite Surface Protein-1 (MSP-1) malaria pseudopeptide analogues containing a Ψ[CH_2–NH] reduced amide isostere were prepared.[15] Each pseudopeptide-induced antibody showed distinct recognition patterns. A (*Z*)-alkene Ser-*cis*-Pro mimic (**12**) was synthesized through the use of a Still-Wittig [2,3]-sigmatropic rearrangement, while an (*E*)-alkene Ser-*trans*-Pro mimic (**13**) was formed using an Ireland-Claisen [3,3]-sigmatropic rearrangement.[16] Samarium diiodide-induced reduction of γ-acetoxy-α,β-enoates led to the preparation of (*E*)-alkene dipeptide isosteres.[17] An (*E*)-alkene dipeptide isostere containing purely nonpeptidic HIV protease inhibitor has been developed.[18] Novel, potent, fluoroolefin peptide isosteres (**14** and **15**) inhibited dipeptidyl peptidase IV competitively

with K_i values of 7.69 and 6.03 μM, respectively.[19] Another dipeptidyl peptidase IV inhibitor, compound **16**, was synthesized with a Wadsworth-Horner-Emmons reaction followed by amide formation and reduction of the amide.[20] The total synthesis of the aspartyl protease inhibitors L-682,679, L-684,414, L-685,434, and L-685,458 involving an allyltrichlorostannane coupling with an α-amino aldehyde has been reported.[21] Three diastereomeric hydroxyethylene isosteres of the Val–Ala dipeptide having the general formula of (**17**) were synthesized from α,β-unsaturated ketones.[22] Symmetric and asymmetric Ψ[CH(OH)–CH_2] peptidomimetics were found to inhibit both HIV-1 and *Candida albicans* aspartic proteases.[23] A novel biotinylated affinity ligand containing a hydroxyethylene dipeptide isostere was developed to generate a molecular probe for γ-secretase.[24] Ketomethylene peptide isosteres have been prepared through a zinc-mediated chain extension reaction in which amino acid-derived β-keto esters were converted to γ-keto esters in a single step.[25] An X-ray structure of mutant and wild type HIV-1 proteases complexed with Boc–Phe–Ψ[CH_2CH_2NH]–Phe–Glu–Phe–NH_2, the newly developed peptidomimetic inhibitor OE, gave experimental evidence that the ethylenamine isostere bonded tightly to both HIV-1 proteases.[26] Novel HIV protease inhibitors containing a hydroxyethylamine dipeptide isostere (HDI) as a transition state mimic were synthesized.[18] TYA5 (**18**) and TYB5 (**19**) were proven to be not only potent enzyme inhibitors (K_i = 0.12 nM and 0.10 nM, respectively) but also strong anti-HIV agents (IC_{50} = 9.5 nM and 66 nM, respectively). HDI structure containing β-secretase inhibitors were synthesized for the first time.[27] Some of the compounds showed inhibitory activity in the nanomolar range. The first solid-phase synthesis of Ψ[CH_2(OH)–CH_2–NH] isostere of human T-cell leukemia virus type-1 (HTLV-1) protease inhibitors was published.[28] A new succinic acid linker was developed, which enables an efficient preparation of the inhibitors on the solid support.



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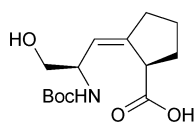

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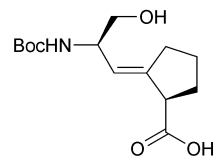

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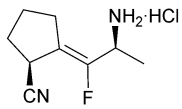

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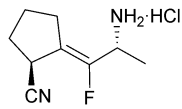

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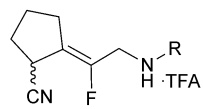

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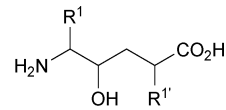

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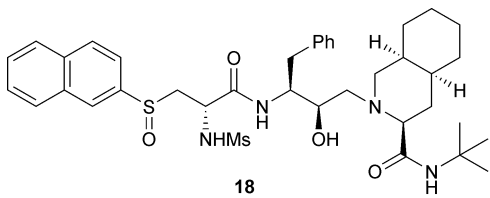

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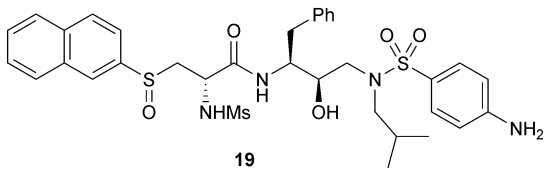
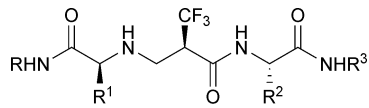


19

A 109-reference report reviewed the solution-phase chemistry, structural and conformational investigations of partially-modified retro- and retro-inverso Ψ[NHCH(CF_3)]Gly peptides.[29] In spite of the lack of intramolecular hydrogen bonding, turn-like conformation was found for most of the pseudopeptides in which the CF_3 group points towards the outside position of the turn itself. A highly stereoselective synthesis of partially-modified retro-Ψ[$NHCH_2$]-peptides that incorporate a trifluoroalanine mimetic was described.[30] The same research group published the stereocontrolled synthesis of Ψ[NHCH(CF_3)]Gly-peptides using a kinetically controlled aza-Michael addition of chiral α-amino esters to *trans*-3,3,3-trifluoro-1-nitropropene.[31] A library of partially-modified retro-Ψ[$NHCH_2$] peptides having the general formula of (**20**) was synthesized in a stereocontrolled manner by means of a tandem asymmetric aza-Michael/enolate-protonation.[32]



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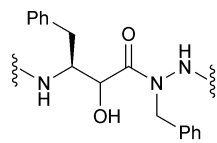
Retro-inverso analogue of the antiviral octapeptide C8 (Ac–D-Ile–D-Trp–Gly–D-Val–D-Trp–D-Asp–D-Glu–D-Trp–NH_2) maintained conformational features believed to be important for antiviral activity, and displayed a remarkable serum stability.[33] Retro-inverso analogues of model T and B cell epitopes were designed and assayed for activity.[34] While the T cell epitopes could be mimicked, a retro-inverso analogue of an immunodominant B cell epitope exhibited no structural or functional correlation with its natural counterpart. Retro-inverso gonadotropin-releasing hormone (GnRH) peptide elicited high titers of anti-GnRH antibodies in rabbit and mice.[35] Six pseudohexapeptide analogues of the human leukocyte elastase (HLE) substrate *Z*–Ala–Ala–Pro–Val–Ala–Ala–NH*i*Pr in which one of the amide bonds is replaced by amidoxy Ψ[CO–NH–O], aldoxime Ψ[CH═N–O] and hydroxylamine Ψ[CH_2–NH–O] surrogate have been synthesized and tested for their recognition by HLE.[36] Pseudo-symmetric HIV-1 protease inhibitors containing a novel hydroxymethylcarbonyl (HMC)-hydrazide isostere (**21**) were designed and synthesized.[37] Most of the compounds showed potent inhibitory activity with nanomolar K_i values. Isocyanates could be prepared readily from Fmoc-amino acid azides.[38] Isocyanates could be coupled with *N*,*O*-bis(dimethylsilyl) derivatives of amino acids to obtain urea peptide derivatives having the general formula of (**22**).



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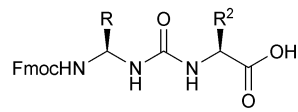

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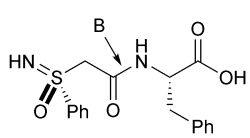
While the sulfoximine-pseudopeptide modification in compounds (**23**) and (**24**) stabilized the following peptide bond B against enzymatic cleavage, the pseudopeptide bond A in compound (**24**) was readily cleaved by the enzyme.[39] New phosphinopeptidic building blocks containing a triple bond (**25**) were synthesized in solution phase.[40] A post-assembly 1,3-cycloaddition of these pseudopeptides led to a novel class of isoxazole-containing phosphinic peptides (**26**). Another isoxazole-containing phosphinic tripeptide array (**27**) was synthesized by a solid-phase strategy. Some of these pseudopeptides were proved to be potent matrix metalloprotease inhibitors (K_i in the low nanomolar range). Enantioselective anion-exchange employing quinidine and quinine carbamate chiral stationary phases were used for the enantiomeric separation of phosphinic pseudopeptides as well as their α-aminophosphinic acid precursors.[41] A new method which combines affinity chromatography and combinatorial chemistry was developed to identify protein targets for phosphinic pseudopeptide ligands.[42]

Characterization of phosphinic pseudopeptides by capillary zone electrophoresis in highly acidic background has been published by the same research group.[43]



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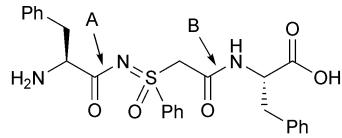

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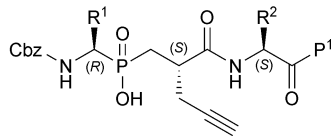

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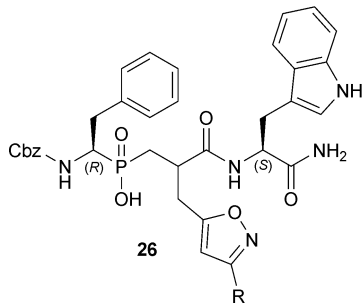

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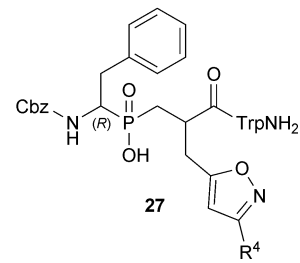

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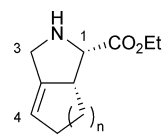
2.3 Rigid amino acid, peptide and turn mimetics

Synthetic efforts for the preparation of cyclic constrained Phe analogues using the building block approach have been reviewed.[44]

The symmetrical building blocks were prepared by dialkylation of ethyl isocyanoacetate while the unsymmetrical building blocks were prepared by a stepwise alkylation of the O'Donnell Schiff base. Direct addition of aryl radicals to the nitrogen of azomethines proved to be an efficient, synthetically useful process for amination of an aromatic ring.[45] The free radical-mediated amination sequenced with the O'Donnell phase transfer-catalyzed enantioselective alkylation of glycinyl imine provided indoline α-amino acids as constrained Phe derivatives. A new strategy for the synthesis of bicyclic analogues of both L- and D-proline was developed.[46] The scaffolds were prepared as Fmoc-amino acids suitable for solid-phase peptide synthesis. A highly selective asymmetric synthesis of new unsaturated fused bicyclic proline analogues (**28**) has been described.[47] The key steps of the synthesis are the highly region- and diastereoselective alkylation of cyclic bis(allylsulfoximine)titanium complexes with *tert*-butylsulfonyl imino ester and a novel migratory cyclization of δ-amino alkenyl sulfoxonium salts. An asymmetric synthesis of fused bicyclic amino acids (**29**) having a hexahydro-cyclopenta[*c*]pyridine skeleton and an enone structural element was published.[48]



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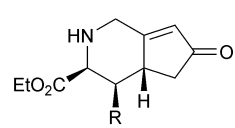

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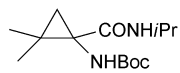
Diverse bicyclic sulfonamides as constrained proline analogues were synthesized and applied to the design of thrombin inhibitors.[49] Beta-substituted

conformationally constrained prolines with the 7-azabicyclo[2.2.1]heptane scaffold have been prepared in an enantiomerically pure form.[50] *N*-Protected derivative of (*R*)-c_3Val (1-amino-2,2-dimethylcyclopropane-1-carboxylic acid) (**30**) was synthesized and incorporated into the model peptides *t*BuCO–L-Pro–L-c(3)Val–NH*i*Pr and *t*BuCO–L-Pro–D-*c*(3)Val–NH*i*Pr.[51] Both dipeptides accommodated a type II β-turn. Model peptides, containing one or two (*R*)-c_3Val residues were prepared in combination with either Aib or Gly residues.[52] An efficient method for the preparation of a conformationally constrained β,γ-diamino acid derivative (**31**) have been developed.[53] The synthesis involved chemoselective enzymatic hydrolysis of *cis*-piperidine-3,5-dicarboxylic ester followed by resolution of the half-acid. The optically active half-acid was transformed to the cyclic amino acid *via* Curtius-rearrangement.



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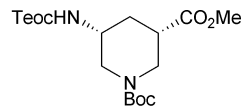

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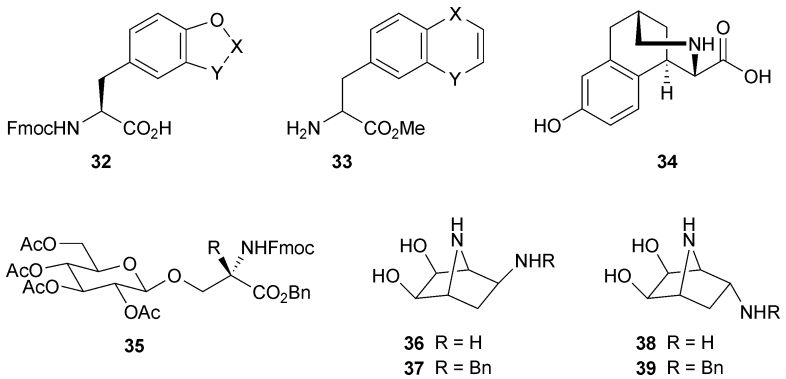
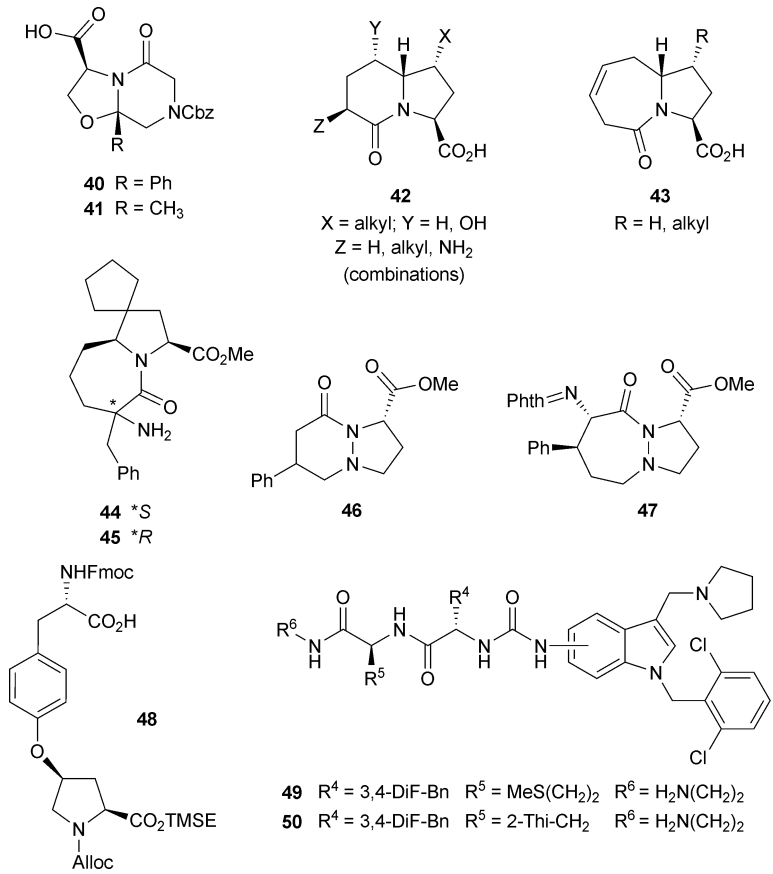
Conformational constraints have been prepared by insertion of either *cis*- and *trans* double bonds or tricyclic ring structures in dicarba cystine analogues.[54] A mild and general strategy for the synthesis of 2-substituted-4-amino-1,2,4,5-tetrahydro-2-benzazepine-3-ones as constrained phenylalanine derivatives was published.[55] The synthesis uses the amide bond formation for cyclisation. A practical synthesis of α,α-diisobutylglycine *via* Pd-mediated diallylation of α-nitroacetate has been reported.[56] The product was coupled to PAL-linker on PEG-PS resin in a sufficient way. A series of *N*-substituted glycine oligomers (peptoids) and peptide–peptoid hybrids based on the Ac–His–Phe–Arg–Trp–NH_2 tetrapeptide were synthesized and tested at the mouse melanocortin receptors for agonist activity.[57] Effective site-specific incorporation of sterically demanding α-fluoroalkyl amino acids into the P_1 position of peptides catalyzed by trypsin and α-chymotrypsin was published.[58] Phenylalanine derivatives as phosphotyrosine mimetics having the general formulae (**32** or **33**) were designed and synthesized.[59] The mimetics were successfully incorporated *via* solid-phase synthesis into a library containing triazolopyridazine β-strand templates. A novel phosphatase-stable β-amino-phosphotyrosine mimetic was utilized to prepare previously reported olefin-metathesis-derived macrocycles in another study.[60] Three nonhydrolyzable phosphotyrosine mimics that contain α-ketoacid, α-hydroxyacid, and methylenesulfonamide groups in place of the phosphate were incorporated into the peptide sequence Ac–Asp–Ala–Asp–Glu–X–Leu–NH_2, where X is the pTyr mimic, and analyzed against *Yersinia* PTPase and PTP1B.[61] An enantioselective synthesis of a new tricyclic tyrosine analogue (**34**) was developed.[62] The preparation utilized a tandem asymmetric Michael-addition/substitution reaction, intramolecular Friedel-Crafts reaction, and intramolecular Mannich reaction as key steps. A suitably protected β-D-glucopyranosyl-(*S*)-α-methylserine derivative (**35**) as a novel conformationally restricted analogue of β-D-glucopyranosyl-L-serine was synthesized.[63] An efficient approach to the synthesis of conformationally constrained bicyclic 1,2-diamines (**36–39**) has been described.[64] The enantiomerically pure analogues were evaluated as glycosidase inhibitors.

Alkyl 4-alkyl-2-hydroxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylates are peptidomimetic building blocks for the synthesis of integrin receptor antagonists and serine protease inhibitors.[65] A general synthetic route for the preparation of these compounds was described. Constrained dipeptide scaffolds (**40**) and (**41**) bearing the oxazolo[3,2,*a*]pyrazin-5-one core structure have been synthesized.[66] Cyclocondensation of 3-aza-1,5-ketoacids and aminoalcohols enabled single-step formation of both rings in a highly stereoselective manner. Two versatile methods for the preparation of functionalized enantiopure 1-aza-2-oxobicyclo[4.3.0]nonane carboxylic acid (**42**) and the analogues [5.3.0]decenecarboxylic acid (**43**) have been



reported.[67] A constrained peptidomimetic thrombin inhibitor which was synthesized using one of the bicyclic indolozidinones showed nanomolar activity (IC_{50} = 4.7 nM). New bicyclic lactam peptidomimetics, compounds (**44**) and (**45**), were prepared using ring-closing metathesis reactions.[68] The same research group reported a series of rigid 6,5- and 7,5-fused bicyclic lactams as dipeptide mimics.[69] These substituted 2-oxo-1-azabicycloalkane amino acids could replace the backbone geometry or side-chain function of several dipeptide residues. Searching of cholecystokinin receptor ligands, 1,4-benzodiazepine derivatives have been constructed. Stereocontrolled synthesis of 2-substituted 5-phenyl-1,4-benzodiazepines involved, as a key step, a one-pot cyano reduction and reductive cyclization of the appropriate amino nitrile.[70] Alpha-amino nitriles were involved also in the preparation of 2-substituted 5-oxo-1,4-benzodiazepines.[71] One of these tryptophan-derived mimetics showed nanomolar binding affinity at CCK_1 receptors. Dipeptide mimetic 6,5-fused bicyclic thiazolidinlactams showing selective synthetic transformations of hydroxy groups, excellent solubility, and a tendency to form crystals have been synthesized from a single bicyclic precursor that is accessible in large quantities.[72] Conformationally constrained (*R*)-Pro-(*S*)-Pro peptidomimetics were the products of the thermal and LiBr-DBU catalysed cycloadditions of imines derived from (1*S*,9*S*)-*t*-butyl-9-amino-octahydro-6,10-dioxo-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylate.[73] Pyrazole derivatives as proline surrogates were incorporated into constrained X-Pro peptidomimetics.[74] The synthetic route allowed for the preparation of dipeptide building blocks having either a six (**46**) or a seven-membered-ring (**47**) annulated onto the pyrazole moiety.

The first enantioselective synthesis of a phenylalanine-based tetrahydropyridazinone and its conversion to the 2-oxo-1,6-diazabicyclo[4.3.0]nonane-9-carboxylate dipeptido-mimetic has been reported.[75] The Miller cyclization of hydroxy hydroxamates was expanded to the synthesis of new 3,6-disubstituted-1,4-diazepan-2,5-diones that can be used as new scaffolds for combinatorial chemistry and as conformational constraints for short peptides.[76] Preparation of 3-amino-δ-valerolactams as X-Gly constrained pseudopeptides through conjugate addition and Curtius rearrangement has been published.[77] Conformation of the Trp–Gly surrogates was investigated by NMR analysis. Dipeptides consisting of a diamino acid and threonine were the starting materials of the synthesis of a new class of rigid fused imidazole ring containing dipeptidomimics.[78] Cyclization of pipecolic acid derivatives led to 4-substituted indolizidin-9-one amino acids as peptidomimics.[79] Suitably protected (2*S*,6*S*,8*S*)-indolizin-9-one amino acids were synthesized the first time.[80] A tyrosine–proline peptidomimetic scaffold, compound (**48**) was the base for a 1728-member combinatorial library which led to the discovery of new inhibitors of TNF-α induced apoptosis.[81] Indole-based peptide mimetics, such as compounds (**49**) and (**50**), proved to be potent thrombin receptor (PAR-1) ligands having the binding affinities of 25 and 35 nM, respectively.[82]



A series of 2,4,5-trisubstituted tetrahydropyran derivatives (Fig. 1) as peptidomimetic scaffolds for melanocortin receptor (MCR) were prepared.[83] The key steps of the synthesis involved a palladium-mediated cross-coupling reaction of a dihydropyran-4-one moiety followed by region- and diastereoselective reduction of sp^2 carbon atoms. Further development of suitably substituted tetrahydropyrans led to the discovery of more potent MC4R and MC1R ligands.[84] Conformationally constrained and flexible pseudopeptide derivatives of the tripeptide pTyr–Val–Asn were designed and prepared as potential antagonists of interactions of phosphotyrosine peptides with the Grb2-SH2 domain.[85] Another research group developed potent Grb2-SH2 domain antagonists that do not rely on phosphotyrosine or its mimics.[86] The most potent peptidomimetic showed binding affinity with an IC_{50} of 75 nM. A 2-oxo-1,3-oxazolidine-4-carboxylic acid as a new, conformationally restricted building block for the construction of pseudopeptides has been designed.[87]

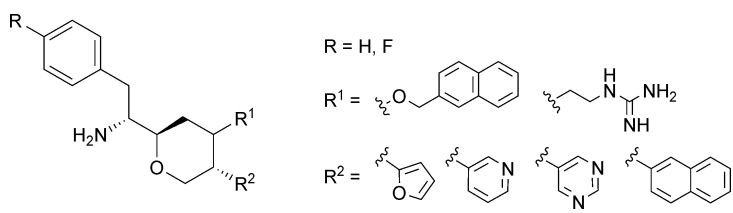


Fig. 1

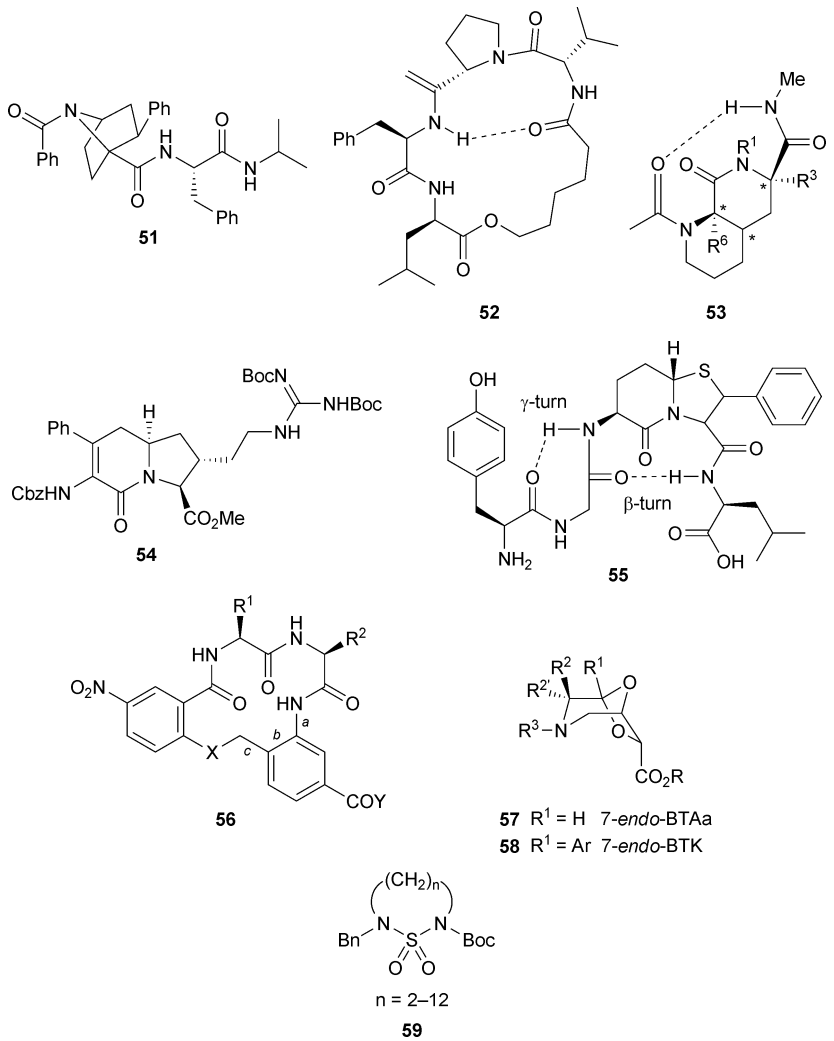
Three-dimensional structure of the homo-oligomers of its (4*S*,5*R*)-5-methyl derivative has been also investigated and showed promise as a template for different applications.

Beta-turn mimicking strategies and their application in the design of potent peptide analogues have been summarized in a 131-reference review.[88] Constrained peptides containing the 2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate (IBTM) system, a dipeptide surrogate of type II′ β-turns were synthesized on the solid-phase.[89] The IBTM moiety was formed *via* a Pictet-Spengler reaction. Two enantiomers of β-substituted ω-unsaturated amino acids have been prepared on a large scale and used for the synthesis of [4,3,0]-bicyclic β-turn mimetics.[90] This strategy allows the introduction of side chain groups with predetermined chiralities. A highly constrained analogue of L-proline, (1*S*,2*S*,4*R*)-2-phenyl-7-azabicyclo-[2.2.1]heptane-1-carboxylic acid, adopted type I β-turn in the model dipeptide **51**.[91] Ring-closing metathesis reaction was used for the synthesis of compound **52**, a novel *cis*-proline derived cyclic mimic of a type VI β-turn.[92] Conformation of the peptide was investigated by NMR. Another type VI β-turn mimic (**53**) was constructed using a substituted perhydro-1,7-naphthyridine ring system.[93] Detailed NMR analysis and molecular modelling were applied to examine conformational behavior of compound **53**. According to FT-IR and NMR investigations, oxanipecotic acid dimers proved to be more stable turn motifs than nipecotic acid dimers.[94] Alpha-aminooxy tripeptides consisting of oxanipecotic acid dimer and α-aminooxy acid adopted unusual folded structures with consecutive β- and γ-turn-like conformations.[95] The novel dipeptide β-turn mimetic, 4,8-disubstituted azabicyclo[4.3.0]nonane amino acid ester (**54**) was synthesized and used as a peptide mimetic of the dipeptide Phe–Arg of melanotropin peptides.[96] Well-defined turn structures were adopted by aminooxy-acid containing tetramers and a pentamer which were designed as α-MSH analogues.[97] The synthesis of the 1-azabicyclo[5.2.0]-nonan-2-one lactam as a non-peptidic scaffold mimicking the RGD β-turn topology has been reported.[98] Functionalized 2-oxo-1-azabicyclo[5.3.0]alkane β-turn mimetic, a potential scaffold for targeted chemotherapy strategies, was prepared in a stereoselective manner.[99] Four individual isomers of Leu-enkephalin analogues (**55**) have been prepared by incorporating novel β-turn dipeptide mimetics, 8-phenyl thiaindolizidinone amino acids into Leu-enkephalin peptides replacing the Gly–Phe unit.[100] Efficient solid-phase synthesis of constrained 14-membered ring β-turn peptidomimetics (**56**) was published.[101] Reverse turn inducer dipeptide isosteres, enantiopure 7-*endo*-BTAa (**57**) and 7-*endo*-BTK (**58**), were prepared from erythrose.[102] NMR and X-ray analyses revealed that a D,L-α-aminoxy acid dimer induced a novel reverse turn structure in peptides.[103]

3. Cyclic peptides

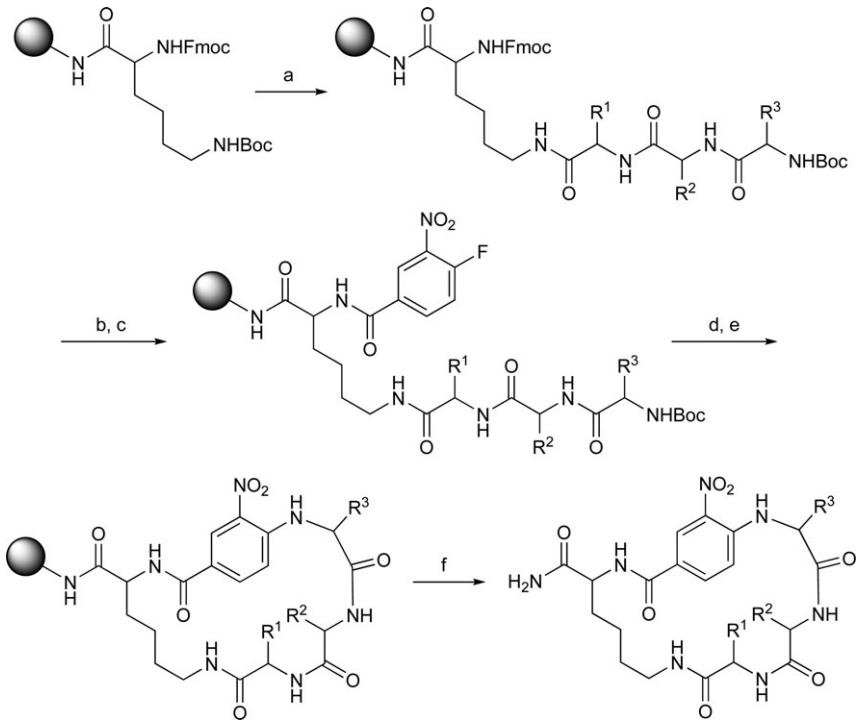
As in last year's chapter in volume 35, the comprehensive coverage of this topic can be seen in Chapter 4. In addition to the discussion of new synthetic routes, this subsection is confined to cyclic pseudopeptides and peptidomimetics.

Dehydro-Freidinger lactams which are cyclic dipeptide mimics were prepared from natural amino acids.[104] The synthesis involved ring-closing metathesis reaction promoted by ruthenium-based catalysts. Unsaturated nine-membered lactams were prepared by an Ugi multicomponent reaction followed by highly selective ring-closing metathesis.[105] A general synthesis of cyclic sulfonamides has been described.[106] The products have been applied in the preparation of pseudo peptides such as compound **59**. Two small cyclic peptides were synthesized *via* intramolecular Heck reactions.[107] Cyclization occurred on tripeptides which contain a 3-bromobenzyl group at the C-termini and an acryloyl group at the *N*-termini. Macrocyclic structures were yielded from U-turn preorganized peptidomimetic molecules and *meta*- and *para*bis(bromomethyl)benzene in acetonitrile.[108]



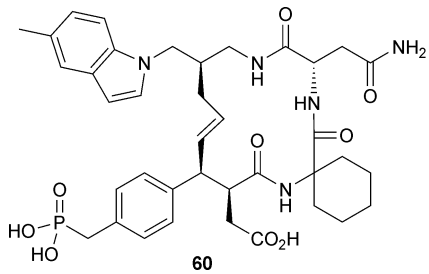
Novel Gly building blocks having the general formula of Fmoc-N^{α}[CH(R)-CO_2Al]Gly–OH have been prepared by the reductive alkylation of allyl esters of several amino acids.[109] The *N*-alkylated Gly units were incorporated into model backbone cyclic peptides. Synthesis, chemical and enzymatic properties of new β-strand mycrocyclic peptidomimetic analogues containing α-(O-, S- or NH-)aryl substituted residues was reported.[110] Macrocycles were synthesized by intramolecular nucleophilic aromatic substitution on solid-support as shown in Scheme 1.[111] The described method can be used to generate libraries of macro-heterocycles. Cyclic peptides containing oxazole and thiazole heterocycles have been visualized by molecular modeling techniques.[112] The examination suggested that these constrained cyclic peptides can be used as scaffolds to create novel protein-like supramolecular structures. The first synthesis of Mannich base bridged cyclopeptides by both solution- and solid-phase protocols was reported.[113]

A phage-library derived non-phosphorylated cyclic peptide ligand of Grb-SH2 domain, cyclo(CH_2CO–Glu–Leu–Tyr–Glu–Asn–Val–Gly–Met–Tyr–Cys)-amide (termed G1TE) provides a novel template for the development of chemotherapeutic agents for the treatment of erbB2-related cancer.[114] A novel 5-methylindolyl-containing macrocyclic tetrapeptide mimetic (**60**) that binds to Grb2 SH2 domain



Scheme 1 . *Reagents and conditions*: (a) Boc SPPS; (b) 20% piperidine in DMF; (c) 4-fluoro-3-nitrobenzoic acid, DIC; (d) 55% TFA in DCM; (e) 5% DIEA in DCM; (f) HF/anisole, 90 min.

with K_d = 75 pM was presented.[115] A cyclic peptide containing a 21-residue epitope of the A–B loop of human immunoglobulin E Cε3 domain has been published.[116] To form the 65-membered ring, an "on-resin" Sonogashira coupling reaction which concomitantly installed a diphenylacetylene amino acid conformational constraint within the loop was used. A macrocyclic, peptidomimetic inhibitor (**61**) of peptide deformylase (PTD) was designed by covalently cross-linking the P1′ and P3′ side chains.[117] The cyclic inhibitor is highly potent against *Escherichia coli* PTD and has antibacterial activity against both Gram-positive and Gram-negative bacteria.



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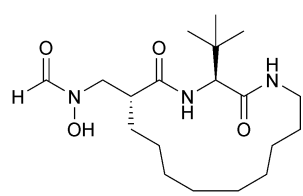

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4. Biologically active peptides

4.1 Peptides involved in Alzheimer's disease

β-Amyloid (Aβ) peptides, containing 39–43 amino acids, play a central role by initiating neurodegeneration in Alzheimer's type dementia. Aβ 1–40, 1–42 and 1–43 peptides are also present in the brain of patients with Down's syndrome.[118] Aβ peptides are synthesized in the brain from β-amyloid precursor protein (APP) by

enzymic cleavage. The two enzymes participating in APP-metabolism and biosynthesis of Aβ peptides (aspartyl-proteases: β- and γ-secretase) are target proteins for drug design (see this chapter 5.15). Aβ peptides can be degraded *in vivo* by different peptidases and proteases.[119,120] Insulin-degrading enzyme (IDE) regulates the level of Aβ-peptides, APP and insulin.[121]

For initiation of Alzheimer's disease the conformational change and aggregation of Aβ peptides seems to be the most important event. The aggregation process has been reviewed with the focus of cholesterol influence on interactions between Aβ peptides and cell membranes.[122] Aβ peptides have different tendency for aggregation and fibril formation, the hydrophobicity of the sequence plays important role in aggregation.[123]

Aggregation of Aβ peptides results in formation of oligomers, protofibrils and fibrils; protofibrils have already a "fibrous crystal" core structure resistant to hydrogen exchange.[124] Thermodynamic calculations show that Aβ 1–40 is thermodynamically soluble at physiological concentrations.[125] Hydrogen-exchanged mass spectrometry has been used for the analysis of Aβ peptide structure.[126] The kinetics of Aβ-aggregation can be modeled as an autocatalytic reaction.[127] The spontaneous aggregation of Aβ 1–40 and the cytotoxicity of the aggregates were studied using a kinetic model.[128] Aggregation of Aβ peptides is pH-dependent; Aβ association kinetics was studied at endosomal pH.[129] Toxic aggregation is influenced also by the specific amino acid sequence of Aβ peptides.[130,131] Aβ 1–40 and 1–42 peptides were identified from mixed pre-fibrillar aggregates.[132] Aβ 1–42 shows a self-assembly into globular forms which are also neurotoxic.[133]

Several peptidic and non-peptidic substances modulate Aβ aggregation: cholesterol in the membranes,[122,134] humanin peptides[135] and surface-tension modifying peptides.[136] Human acetyl-cholinesterase also induces Aβ-aggregation.[137] Amyloid fibril formation can be inhibited by cationic peptides.[138] Novel methods are used for *in vitro* characterization of conditions for oligomerization and fibrillogenesis of Aβ-peptides[139] and a new easy and practical assay was published for studying the molecular mechanism of Aβ aggregation inhibitors.[140] Neurotoxic Aβ mutant peptides cause cerebral amyloid angiopathy and play important roles in the pathogenesis of Alzheimer's disease.[141]

The possible applications of synthetic peptides in the diagnosis of neurological diseases such as Alzheimer's disease have been reviewed.[142] Core biological marker candidates for diagnosis of Alzheimer's disease are reviewed.[143] Application of cerebrospinal fluid (CSF) biomakers (Aβ peptide aggregates, tau-proteins, *etc.*) for disease stage and intensity in cognitively impaired patients has been published,[144] CSF levels of total-tau, hyperphosphorylated-tau and Aβ 1–42 predict development of Alzheimer's disease in patients with mild cognitive impairment.[145] The level of Aβ 1–42 in CSF shows good correlation with amyloid-neuropathology according to a population-based autopsy study.[146]

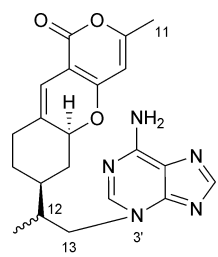
Visualization of β-amyloid aggregates and amyloid plaques in the brain could serve as a diagnostic tool for Alzheimer's disease. The methods of neuroimaging and early diagnosis of Alzheimer's disease[147] as well as a general, non-oncological application of radiolabeled peptides in nuclear medicine are reviewed.[148] Early diagnosis and treatment will be very important in prevention of Alzheimer's disease.[149] *In vivo* labelling of amyloid plaques with an improved thioflavin-T derivative (IMPY)[150] might be applied in the Alzheimer's diagnostic. Iodinated tracers could be also applied for imaging amyloid plaques in the brain.[151–153] Pozitron emission tomography (PET) applying ^{11}C-labeled stilbene derivatives[154] or ^{11}C 6-substituted 2-arylbenzothiazoles[155] as imaging agents specific for β-amyloid aggregates might be a practical method for diagnosis of Alzheimer's disease. *In vitro* detection of plaques in the Alzheimer's brain using PET molecular imaging probe 2-(1-[6-[(2-[(18)F]fluoroethyl)(methyl)amino]2-naphthyl]ethylidene)malonitrile has been also published.[156] The synthesis of a potential position-labeled probe for imaging β-amyloid aggregates is described,[157] the compound is a ^{18}F-labeled, 3-(2-fluoroethyl)ethylamino-6-diethyl-aminoacridine. Other substances, such as

2-dialkylamino-6-acylmalonitrile substituted naphthalenes[158] are published as novel diagnostic and therapeutic tools in Alzheimer's disease.

The biological effects of β-amyloid peptides were further studied. Aβ peptides induce the biosynthesis of matrix-degrading proteases in cultured rat astrocytes[159] and rapidly inhibit fast axonal transport in rat hippocampal neurons.[160] Aβ 1–42 peptide increases acetylcholinesterase expression in neuroblastoma cells by reducing enzyme degradation.[161] The pro-inflammatory effects of Aβ 1–42 can be inhibited with pravastatin in glioma cell culture.[162] Aβ peptides potentiate inflammatory responses induced by lipopolysaccharide, γ-interferon and advanced glycation endproducts in a murine microglia cell line.[163] It is interesting that α7 nicotinic acetylcholine receptors show different physiological responses to Aβ 1–40 and Aβ 1–42.[164]

Vaccination for preventing Alzheimer's disease might be an important method in the future. Inflammation and therapeutic vaccination in central nervous system diseases are reviewed.[165] Conformation, aggregation and biological activity of Aβ peptide fragments have been studied. Aβ 25–35 induces apoptosis in a neuroblastoma cell line.[166] The conformation, structure and texture of fibrous crystals formed by Aβ 11–25 peptide fragment were studied.[167] X-ray diffraction studies of Aβ 25–35 and Aβ 31–35 peptide assemblies show reverse-turn conformation and side-chain interactions in the aggregates.[168]

The prevention of neurotoxic actions of Aβ peptides has been intensively studied. The sexual hormone estradiol prevents Aβ peptide induced cell death in a cholinergic cell line *via* modulation of a classical estrogen receptor.[169] According to preliminary studies, estradiol reduces the plasma level of Aβ 1–40 for postmenopausal women with Alzheimer's disease.[170] Non-steroidal anti-inflammatory drugs modulate Aβ-metabolism in neuronal cell cultures[171] and chronic treatment with indomethacin rescues learning deficits and dysfunctional synaptic plasticity induced by aggregated Aβ peptides.[172] Aβ toxicity in the PC12 cell line can be inhibited by resveratrol and catechin.[173] On the other hand, red wine micronutrients can serve as protecting agents in Alzheimer-like induced insult.[174] Melatonin also protects neurons against Aβ (and glutamate) toxicity, the mechanism of protection has not yet been proven; GABA receptors might be involved.[175] Conformationally constrained cyclic peptides from Aβ 1–28 are inhibitors of Aβ toxicity.[176] Fig. 2 shows the primary structure of Aβ 1–28 and Cyclo^{17,21}-[Lys^{17}, Asp^{21}]Aβ 1–28. Combinatorial methods were used for studying a new class of one-armed cationic peptides targeting the C-terminus of the Aβ 1–42 peptide.[177] 4-Deoxy analogs of glucosamine (a precursor of heparin sulfate biosynthesis) are effective anti-amyloid agents both *in vitro* and *in vivo*; some novel glycosaminoglycan precursors were synthesized as new anti-amyloid compounds.[178] These substances inhibit the binding between heparin sulfate and the β-amyloid peptides and thus the conformational change of the peptide and fibril formation. Syntheses and bioactivities of tricyclic pyron derivatives (**62**) that show a significant effect in protecting against neuronal cell death from the intracellular accumulation of Aβ peptides are described.[179]



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Peptide nucleic acids as putative drugs have been synthesized and applied for targeting the biosynthesis of amyloid precursor protein.[180]

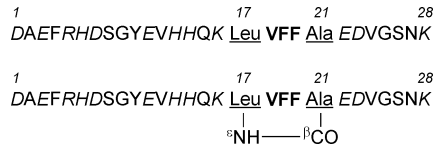


Fig. 2

4.2 Antimicrobial peptides

The number of papers published in the year of 2003 shows that antimicrobial peptides (AMP) attract still increasing interest. Several different aspects of antimicrobial peptides have been reviewed in this year. The main components of the innate immunity: antibacterial peptides we are all 'born with' were summarized in a review showing why most of us stay healthy.[181] Antimicrobial peptides found in domesticated animals including the sites of production and their potential use for therapeutic treatment was the subject of another work.[182] The current knowledge on the basic and applied biology of antimicrobial peptides has been reviewed.[183] The continuing emergence of drug resistance compels the pharmaceutical industry to generate new agents. The genome revolution provided alternative strategies for validating new targets. A study discussed the roles for genetic analyses in the antibacterial drug-discovery process.[184] Novel agents for the treatment of resistant Gram-positive infections were reviewed.[185] Some of these compounds such as daptomycin, oritavancin, dalbavancin and ramoplanin are in advanced stages of development at present. Recurrent structural and functional themes among mechanism of action and resistance observed for antimicrobial peptides of widely diverse source and composition were discussed.[186] This understanding may provide new models and strategies for developing novel antibacterial agents. Another review focussed on the function of lipopolysaccharides as the major constituents of the outer layer of the outer membrane of Gram-negative bacteria and the mechanism of interaction between these membranes and antibacterial peptides.[187] Some of the main types of ribosomally synthesized antimicrobial peptides produced by eukaryotes and prokaryotes, new developments and novel applications related to these peptides have been reviewed.[188] A study provided an overview of antimicrobial peptides of the cathelicidin family, the structures of their genes and peptides and their biological functions.[189] Among others, food proteins can be considered as sources of antimicrobial compounds. Antimicrobial peptides generated through proteolytical digestion of food proteins were discussed.[190] Lactoferrins, milk protein-derived antimicrobial peptides were the subject of another review.[191] A comprehensive coverage of these peptides and their synthetic analogues were presented. Starting from the point of antimicrobial resistance, a review discussed the agents that can be truly regarded as new drugs, namely the oxazolidinones, the cationic peptides and the lipopeptide antibiotics.[192] In addition to these, peptide deformylase inhibitors and pleuromutilins as potential future drugs were discussed, too. Recent isolations of antimicrobial compounds from insects, with molecular masses less than 1 kDa as well as the first data on the antimicrobial properties of a promising dipeptide (β-alanyl-tyrosine) isolated from the fleshfly *Neobellieria bullata* were reported.[193] The article, which is based on the Leonidas Zervas Award lecture given at the 2002 EPS in Sorrento, the progress made in the area of *Staphylococcus aureus* secreted autoinducing peptides was reviewed.[194]

4.2.1 Antibacterial peptides. Synthetic tick defensin demonstrated strong antibacterial activity against Gram-positive bacteria and low hemolytic activity.[195] Investigation of the mechanism of action provided evidence that tick defensin caused cytoplasmic membrane lysis in *Micrococcus luteus*. NP-1, a rabbit defensin,

prevented virally mediated fusion events, entry, and cell-to-cell spread of herpex simplex virus type 2.[196] Cryptdin-4 (Crp4) is the most potent mouse α-defensin *in vitro*. Amino acid substitutions introduced to change the charge and hydrophobicity of the Crp4 N-terminus affected bactericidal activity modestly, apparently by influencing the peptide binding to phospholipids bilayers and subsequent permealization of target cell membranes.[197] Two isoforms of a novel 38-residue AMP, spheniscin, belonging to the β-defensin subfamily were identified and fully characterized.[198] Data suggested that sphenicins might play a role in the long term preservation of stored food in the stomach of king penguins. A cysteine substitution analogue of magainin-2 amide (mag-N22C) having the sequence of GIGKFLHSAKKWGKAFVGEIMC–NH_2, and a disulfide-linked dimer prepared by air oxidation were synthesized and examined.[199] The dimer showed enhanced permeabilization and antimicrobial activity, when compared with the monomeric peptide, particularly at very low concentrations. The results suggest that dimerization of pore-forming, positively charged, amphipathic helical peptides may be a useful general approach to the generation of more potent antimicrobials. A computer model of the magainin pore in a bacterial membrane was constructed to study the molecular basis of magainin selectivity and specificity.[200] Different pathways of bilayer disruption by the structurally related antibacterial peptides cecropin B, B1 and B3 have been identified.[201] On the basis of the observations, models by which these peptides induce lysis of lipid bilayers were discussed. The pathway of the cell membrane lysis of cecropin B was examined by another group using transmission electron microscopic (TEM) examinations.[202] *Ascaris suum* from pig and *Ascaris lumbricoides* from human were found to produce linear (cecropin P1) and cysteine-rich (ASABF) peptides with activity against either Gram-negative or Gram-positive bacteria, respectively.[203] Residues 1–9 of the C-terminal 15-residue segment of melittin when substituted individually, changed the hydrophobicities in these positions and the resulting analogues were tested for antimicrobial and hemolytic activities.[204] The effects of two Phe residues at positions 14 and 15 of CRAMP-18 on structure, antibacterial activity, and interaction with lipid membranes were investigated by Ala substitutions of these amino acids.[205] Both Phe residues were found to be essential for the activity and α-helical structure of the peptides.

In vitro and *in vivo* antimicrobial activities of two α-helical cathelicidin peptides (BMAP-27 and -28) and four synthetic analogues were compared.[206] The parent peptides and the mBMAP-28 analog (GGLRSLGRKILRAWKKYGPQAT-PATRQ–NH_2) protected mice from lethal i.p infections in an acute peritonitis model at peptide concentrations significantly lower than the toxic doses. Calmodulin-binding properties of indolicidin, ILPWKWPWWPWRR–NH_2, a 13-residue AMP having the calmodulin-recognition 1–5–10 hydrophobic pattern were investigated.[207] The results indicated that ability to adopt amphiphilic α-helical structure is not a prerequisite for binding to calmodulin. Structure, protease stability and antibacterial activity of CP-11, ILKKWPWWPWRRK–NH_2, an indolicidin analogue and the disulfide-bonded dimer cycloCP-11, ICLKKWPWWPWRRCK–NH_2, were investigated.[208] In addition to the fact, that cyclization greatly improved protease stability; the antibacterial activity of the dimer of cycloCP-11 in the presence of trypsin was completely retained up to 90 min since the major degradation product was equally active. This paper suggested another indication that cyclization might serve as an important strategy in the design of antimicrobial peptides (see ref. 19). Membrane binding and relative penetration of indolicidin analogues were studied using lipid/polydiacetylene chromatic biomimetic membranes.[209] To gain a better understanding of protegrin-1's (PG-1) mechanism of action with membranes, the outer layer of bacterial and red blood cell membranes were represented with lipid monolayers.[210] It was found that the degree of PG-1 insertion into anionic or lipid A monolayers is much larger than that of zwitterionic ones. The cathelin-like domain of protegrin-3 was overexpressed in *Escherichia coli*

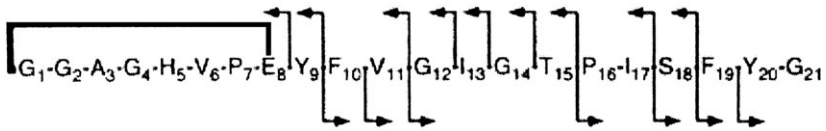
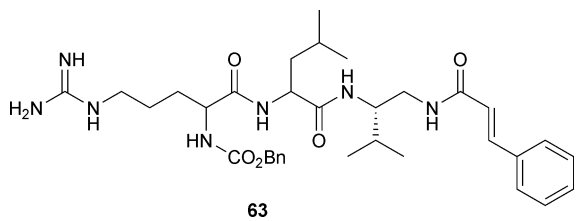


Fig. 3

and labeled with ^{15}N or ^{15}N and ^{13}C isotopes.[211] The three-dimensional structure of the peptides was determined by heteronuclear NMR at pH 6.2.

Acyl analogues of the peptide fragment of human lactoferrin were prepared.[212] Twelve carbon units constituted the optimal chain length which enhanced the antibacterial activity and binding of lipopolysaccharide by up to two orders of magnitude. Inclusion of arginine, lysine, tryptophan, or isoleucine residues enhanced effectiveness of bovine lactoferricin against certain bacteria.[213] The high-resolution crystal structure of the bacteriocin AS-48 showed that this bacteriocin was able to adopt different oligomeric structures according to the physicochemical environment.[214] *Propionibacterium thoenii* P-127 is thought to be the first known bacterium which produces two different bacteriocins, namely PLG-1 and GBZ-1, under different growth conditions.[215] The antibacterial peptide microcin J25 (MccJ25) inhibits bacterial transcription by binding within, and obstructing, the nucleotide-uptake channel of bacterial RNA polymerase. It was reported by two research groups independently that, contrary to the published structure, microcin J25 does not have a head-to-tail cyclic structure.[216,217] The results indicate that MccJ25 in fact is a 21-residue "lariat-protoknot", consisting of an 8-residue cyclic segment formed between Glu^8 and the N-terminus. The structure of microcin J25 and its fragments result from double-cleavage are shown in Fig. 3. Another paper proved the importance of two polar residues, carboxyl group of a Glu and side chain of a His, in the antibiotic activity against *Escherichia coli* and *Salmonella newport*.[218] Synthesizing modified analogues, the structure–activity relationship of citropin 1.1, a broad-spectrum antibiotic and anticancer agent that also causes inhibition of neuronal nitric oxide synthase was investigated.[219] The terminal residues were indicated to be important for antibacterial activity of this amphipathic α-helical peptide.

The synthesis and antibacterial properties of a novel cystatine C-based peptidyl derivative, (2*S*)–2–(N^{α}-*Z*-Arg–Leu–NH_2)-1-[(*E*)-cinnamoylamido]-3-methylbutane, (**63**) were described.[220] A convenient method for the solid-phase synthesis of the cyclic cationic peptide polymyxin B1 and analogues was published.[221] The methodology is based on cleavage-by-cyclization using Kenner's safety-catch linker. Gramicidin thioesterase correctly cyclized immobilized linear gramicidin S precursors into head-to-tail products, indicating its suitability for parallel solid-phase synthesis.[222]



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A 102-reference study reviewed the relationship between peptide structure and antibacterial activity and concluded two main requirements for antimicrobial activity, (i) a cationic charge and (ii) an induced amphiphathic conformation.[223] Three-dimensional structure of spinigerin, a linear antibacterial peptide isolated from the fungus-growing termite *Pseudacanthotermes spiniger* was determined by CD and NMR spectroscopy in SDS micelles.[224] The α-helical structure and the

string electrostatic attraction between four Lys and three Arg residues of spinigerin and the negatively charged head groups of the phospholipids on the membrane play important roles in disrupting membrane. CD, FT-IR, fluorescence, and NMR spectroscopy was used to determine the three-dimensional structure of another AMP, dermaseptine B2 in aqueous solution, in TFE/water mixtures, and in micellar and nonmicellar SDS.[225] The key finding of the study is that the structures of this peptide in TFE and in micellar SDS differ significantly. Thus, the peptide helix is able to adopt the proper conformation which allows it to interact optimally with the micelle surface. The connection between the solution structures and activity of caerin 1.1 and caerin 1.4 was investigated in aqueous TFE and dodecylphosphocoline.[226] It was reported that a 13-residue domain of puroindoline A, including its Trp-rich domain (puroA), exhibited activity against Gram-positive and Gram-negative bacteria.[227] In addition, the structure of puroA bound to membrane-mimicking SDS micelles was determined by two-dimensional NMR.

An array of 40 covalently linked vancomycin dimers was designed and synthesized.[228] The array was used to systematically probe the impact of linkage orientation and linker length on biological activity against susceptible and drug-resistant Gram-positive pathogens. The increased antibacterial activity of a fatty acid-modified synthetic antimicrobial peptide of human cathepsin G was explained by (i) determining the secondary structure motifs in the unmodified and modified peptides and (ii) by measuring the capacity of peptides to insert into large unilamellar liposomes.[229] The synthesis and comparison of the antibacterial properties of hydrophobic derivatives of various antibiotics and poorly active degradation products was the subject of a work.[230] Structure–activity relationship of analogues of pleurocidin, an AMP isolated from the skin of the winter flounder, was published.[231] Antibacterial and hemolytic activities of tyrocidine A, an amphiphatic cyclic decapeptide antibiotic, were successfully dissociated using an alanine-scanning method.[232] Dermaseptin S4 and its substituted derivative K_4-S4 were investigated against various food-related pathogenic bacteria in culture media.[233]

The mode of action of many antibacterial peptides is the disruption of the plasma membrane. Investigation of the interaction of antimicrobial peptides and model phospholipids membranes has been reviewed.[234] It was shown that purified amoebapores, archaic effector peptides of protozoan origin, killed bacteria and that the individual isoforms showed different antibacterial activity against a particular bacterial species.[235] Serum transferring, ovotransferrin and lactoferrin were able to permeate the *Escherichia coli* outer membrane and to access the inner membrane, where they cause permeation of ions in a selective manner.[236] Promising candidates for ''new antibiotics'' act by direct destabilization of the target cell membrane. Molecular basis for the selectivity of the synthetic peptide NK-2 utilizing model systems mimicking the eukaryotic and prokaryotic cell membranes have been investigated.[237] Solid-state NMR and CD spectroscopy were used to study the effect of antimicrobial peptides (aurein 1.2, citropin 1.1, maculatin 1.1 and caerin 1.1) from Australian tree frogs on phospholipid membranes.[238] The behavior of ceratotoxin A, a 36-residue AMP isolated from the medfly *Ceratitis capitata*, in planar lipid bilayers by measuring its pore-forming activity was investigated.[239] The results suggested that ceratotoxin A acted according to the barrel-stave model.

Several antibacterial peptides have been newly isolated from natural sources. Two novel antibacterial peptides of clostridiall species were purified, N-terminally sequenced, and characterized.[240] A 33-residue AMP called dolabellanin B2 was isolated from the sea hare *Dolabella auricularia*'s body-wall including skin and mucus.[241] Acanthoscurrin is a novel glycine-rich AMP from the hemocytes of unchallenged tarantula spider *Acanthoscurria gomesiana*.[242] Extracts of *Styela clava*, a cosmopolitan solitary tunicate, contained small antimicrobial peptides which fell into two distinct families by purification named styelins and clavanins.[243] Isolation of a 51-amino acid AMP from the skin mucus of Atlantic halibut (*Hippoglossus hippoglossus L.*) termed hipposin, its sequence and antibacterial activity were

published.[244] Oncorhyncin III, a novel 6.7 kDa antimicrobial peptide from trout skin secretions exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria, with minimal inhibitory concentrations in the submicromolar range.[245] A study demonstrated that the amino-terminal part of astacidin 1, a newly isolated AMP from the plasma of the freshwater crayfish *Pacifastacus leniusculus*, contributed to the broad-range antibacterial activity.[246] Two clusters containing several pleurocidin-like antimicrobial peptide genes and pseudogenes have been identified from the winter flounder, *Pseudopleuronectes americanus* (Walbaum).[247] Novel antimicrobial peptides, designated *Fa*-AMP1 and *Fa*-AMP2, were purified from the seeds of buckwheat (*Fagopyrum esculentum* Moench).[248] The peptides were active against both bacteria and fungi.

Esculetin-1 is a 46-residue AMP present in skin secreations of *Rana ecsulenta*. The gene coding a variant of ecsuletin-1 was inserted into plant DNA.[249] The antimicrobial peptide was isolated from the intercellular fluids of leaves of transgenic plants, suggesting that it was properly processed, secreted outside cells and accumulated in the intercellular spaces. Gene-encoded antimicrobial peptides that protect the skin of hylid and ranin frogs against microorganisms were processed from a unique family of precursor polypeptides with a unique pattern.[250] Precursors belonging to this family, designated the preprodermaseptin, have a common N-terminal preproregion.

Peptidomimetics containing N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) were subjected to structure–activity investigations.[251] Mono- and dechloronated analogues of the A40926 complex of lipoglycopeptide antibiotics which contains chlorine atoms in amino acids 3 and 6 have been produced using a classical mutagenesis and selection approach.[252] The dechlorinated and one monochloro derivative showed improved antibacterial activity against coagulase negative staphylococci strains. Leu–Lys rich antimicrobial peptides have been investigated. Novel analogues by chain-length deletion and increasing net positive charge and hydrophobicity were designed and synthesized from the sequence of cecropin A (1–8)-magainin 2 (1–12) hybrid peptide (CA–MA).[253] One of the analogues, P5, exhibited potent antimicrobial and antitumor activity. P5 was further modified in another study and the novel analogues were used to investigate the correlation between antimicrobial activity and peptide structure.[254] Antibacterial activities of a homologous series of 8–22-residue LK peptides having different sequences, hydrophobicities and secondary structures were studied.[255] The differences between two basic residues, Arg and Lys, based on tritrpticin sequence, with respect to biological activity, secondary structure and membrane interaction, were investigated.[256] Although there were no remarkable conformational differences between the Arg- and Lys-containing peptides, the Lys-substituted analogues exhibited almost two-fold enhanced antibacterial activity but significantly reduced hemolytic activity. An efficient synthetic route to novel carbazole-linked cyclic and acyclic peptoids that allows the introduction of various basic amino acids has been developed.[257] Some of the analogues showed promising antibacterial activity against *Staphylococcus aureus*. There are several reports on the anti-HIV activities of select antimicrobial peptides. A current report explored, whether peptides derived from HIV-1 envelope glycoproteins could exert antimicrobial activity.[258] Twenty-four peptides showed activity against *Escherichia coli* strains among fifteen-residue peptides spanning the entire sequence of HIV-1 gp120 and gp41. Lentivirus lytic peptides (LLPs) are derived from HIV-1 and have antibacterial properties. LLP derivatives were designed for greater potency against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[259] Four peptides with antimicrobial activity were isolated from the human colon mucosa and identified as ubiquicidin, histone H2B, eosinophil cationic protein, and phospholipase A(2).[260] Using immunodetection and mass spectrometry, LL-37, HNP1-3, and HBD-1 were also identified.

A newly developed pyrrhocoricin derivative, Pip-pyrr-MeArg dimer, containing 4-amino-4-carboxy-piperidine at the amino terminus, an intrachain *N*-methyl-

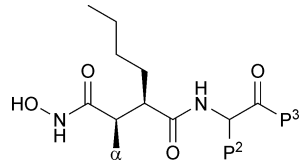


Fig. 4

arginine, and a C-terminal diamino-butyric acid scaffold, killed 11 urinary tract infection-related *Escherichia coli* and *Klebsiella pneumoniae* strains in the sub-low micromolar range.[261] Almost all control antibiotics, including the currently leading trimethoprim-sulfametoxazole combination for urinary tract infection, remained without considerable activity against two or more of these bacterial strains. Imidazolinone derivatives have been described which were the first reported stereochemically discrete MurB inhibitors possessing antibacterial activity.[262] The synthesis and biological activity of analogues of VRC3375 (*N*-hydroxy-3-*R*-3-[(2-*S*-(*tert*-butoxycarbonyl)-pyrrolidin-1-ylcarbonyl]propionamide), an orally active peptide deformylase (PDF) inhibitor were presented.[263] The general structure of succinate hydroxamate analogues can be seen in Fig. 4. Another research group published the synthesis, inhibitory and antibacterial activities of a novel series of benzothiazolylidenehydroxamic acid derivatives as PDF inhibitors.[264]

4.2.2 Antifungal peptides. A review reported on different classes of complex lipids in fungal membranes and on the selective interaction of plant defensins with these complex lipids.[265] Two new cyclic 17-residue peptides, named ranacyclins E and T, were discovered.[266] The first one was isolated from *Rana esculanta* frog skin secretions and the second one was discovered by screening a cDNA library from *Rana temporaria*. Mode of actions and parameters involved in target specificity were also examined. Scarabaecin, a novel cysteine-containing antifungal peptide was isolated from the coconut rhinoceros beetle, *Oryctes rhinoceros*.[267] Chemically synthesized scarabaecin showed activity against several phytopathogenic fungi. Seeds of the green chickpea *Cicer arietinum* were the sources of cicerarin, a new antifungal peptide having the molecular mass of 8 kDa.[268] Cicerarin exerted antifungal activity against *Botrytis cinerea*, *Mycosphaerella arachidicola*, and *Physalospora piricola*. A potent defensin-like antifungal peptide was purified from the seeds of the Yunnan bean and named gymnin.[269] Details of the purification as well as antifungal activity of gymnin were published. The same group reported on the isolation and antifungal activity of cucurmoschin, an 8 kDa-peptide from the seeds of the black pumpkin[270] and of vulgin, a polypeptide from an extract of pinto beans.[271]

It was shown earlier that MUC7 (human salivary low-molecular-mass mucin) 20-mer possessed a broad-spectrum antimicrobial activity. A present study determined the minimum peptide chain length that retains the antifungal activity against *Candida albicans* and *Cryptococcus neoformans*.[272] The C-terminal 12-mer of MUC7 not only retained but exceeded the antifungal activity. Antimicrobial activity, secondary structure, and a possible mechanism of antifungal action of MUC7 were also examined.[273] The short endocytosis signal peptide NPFSD was found to enhance both cellular uptake and toxicity against *Candida albicans* when fused to the ricin A chain toxin.[274] The C-terminal fragment (residues 65–76) of ubiquitin displayed a lytic antifungal activity at the micromolar range.[275] Furthermore, the *N*-(residues 1–34) and C-terminal fragments act synergistically to kill filamentous fungi. Methodology for the enantioselective synthesis of differentially protected *erythro*-α,β-diamino acids from *N*-tosyloxy β-lactams has been reported.[276] The synthetic method was applied to the preparation of an analogue of the antifungal cyclic

mACTH	SYSMEHFRWGKPVGKKRRPVKVYP
K15A;R17A	SYSMEHFRWGKPVGAKARPVKVYP
K16A;R17A	SYSMEHFRWGKPVGKAARPVKVYP
K16A;R18A	SYSMEHFRWGKPVGKARAPVKVYP
K15Q;R17Q	SYSMEHFRWGKPVGQKQRPVKVYP
P19W;K21A	SYSMEHFRWGKPVGKKRRWVAVYP
Ala19-24	SYSMEHFRWGKPVGKKRRAAAAAA
Ala20-24	SYSMEHFRWGKPVGKKRRPAAAAA

Fig. 5

peptide rhodopeptin B5. A series of analogues of bactenecin 7, a cationic antibacterial peptide, was synthesized to investigate the effect of the *N*-terminal configuration on antimicrobial activity.[277] All the synthetic peptides with D-amino acid substitution at the N-terminal showed activity against several fungi at the low micromolar level. Combination of rational drug design and SAR led to the discovery of a potent and selective *Candida albicans N*-myristoyltransferase inhibitor RO-09-4609 [278] which is active against *C. albicans in vitro*. Further modification of this inhibitor resulted in the discovery of a RO-09-4879, which exhibits antifungal activity *in vivo*. NMR spectroscopy and molecular modeling were used to determine the solution structure of both Alo-3, a knottin-type antifungal peptide from the insect *Acrocinus longimanus*[279] and of termicin, an AMP from the termite *Pseudacanthotermes spiniger*.[280]

4.3 ACTH peptides and α MSH analogues

The role of the 15–18 part of the whole ACTH on steroidogenic activity was investigated.[281] The primary structure of mACTH 1–24 and its alanine substituted analogues are shown in Fig. 5. The *in vivo* and *in vitro* experiments led to some important findings concerning the receptor binding and *in vivo* stability of these peptides.

A urea part containing small molecule agonists series of melanocortin receptors were designed and prepared.[282] The structure of these analogues is summarized in Fig. 6.

The receptor specificity of some novel α MSH analogue was discussed in a review.[283] Melanocortin-based analogues can play important role in the regulation of inflammatory processes, nerve regeneration and nociception.

4.4 Angiotensin II analogues and Non-peptide angiotensin II receptor ligands

Tachyphylaxis, defined as the acute loss of response of some smooth muscles upon repeated stimulations with angiotensin II, has been shown to be dependent mainly on the N-terminal region of the ligand. To further study the structural requirements for the induction of tachyphylaxis angiotensin II analogues containing the bulky and lipophilic substituents 9-fluorenylmethyloxycarbonyl and 9-fluorenylmethyl ester at

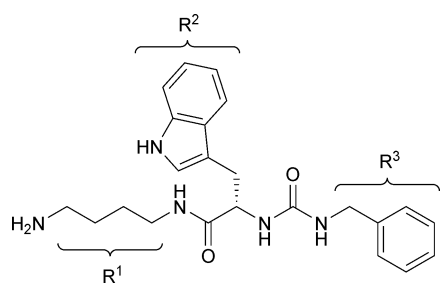
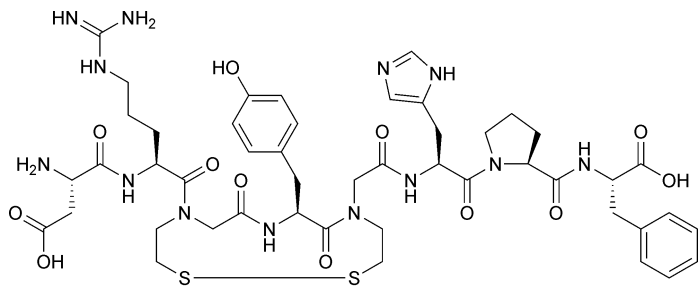


Fig. 6

the Asp[1] residue, have been synthesized.[284] In spite of the lack of a free amino group, the two analogues induced tachyphylaxis. Based in these findings and those available from the literature, an alternate molecular interaction mode between angiotensin II N-terminal portion and the AT1 receptor is proposed to explain the tachyphylactic phenomenon.

N-(2-Mercaptoethyl)glycine (NMGly) was incorporated into the 3 and 5 positions of angiotensin II and oxidized to give the corresponding cyclized disulphide c[NMGly[3,5]]angiotensin II (**64**).[285] The binding affinity to the angiotensin II receptor of this conformationally constrained analog, which is related to the potent angiotensin II agonist c[Hcy3,5]angiotensin II, was examined. The analogue had no affinity to the AT1 receptor.



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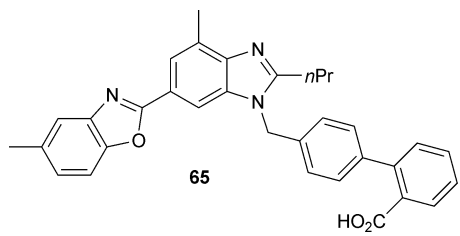
The effects of sarmesin [Sar[1]Tyr(OMe)[4]]angiotensin II (ANG II) on the seizure susceptibility, memory activity and nociception have been studied.[286] This octapeptide, administered i.c.v., dose-dependently decreased the seizure intensity, impaired the memory upon re-testing of rats 24 h later in the passive avoidance test and decreased the pain threshold. Taken together, these results reveal sarmesin as a behaviorally active peptide in the studied experimental animal models. These findings suggest that the methylation of the hydroxyl group of the tyrosine in position 4 of [Sar[1]]angiotensin produces an active analogue that induces a decrease of the seizure susceptibility, an impairment of the memory consolidation and a decrease of the pain threshold.

[Sar[1]Gly[8]]angiotensin II was found as a selective AT_1 receptor antagonist.[287] The difference in its binding affinity to putative AT_{1A} and AT_2 and putative AT_{1B} to AT_2 receptors was 40- and 82-fold, respectively.

1-(Arylmethyl)-6-(methyloxazolyl)-4-methyl-2-propylbenzimidazoles such as (**65**) are prepared as analogues of the angiotensin II receptor antagonist Losartan.[288] For example, compound **65** is prepared in ten steps from 3-methyl-4-nitrobenzoic acid.



65

The endogenous angiotensin II and the synthetic AT2 selective agonist (4-aminoPhe[6]-angiotensin II) respond very differently to identical cyclizations.[289] Cyclizations of angiotensin II by thioacetalization, involving side chains of the amino acid residues 3 and 5, provided ligands with almost equipotent binding affinities to angiotensin II at the AT2 receptor. In contrast, the same cyclization procedures applied on the AT2 selective 4-aminoPhe[6]-angiotensin II delivered significantly less potent AT2 receptor ligands, although the AT2/AT1 selectivity

was still very high. The fact that different structure–activity relationships are observed after imposing conformational restrictions on angiotensin II and 4-aminoPhe^6-angiotensin II, respectively, suggests that these two peptides, despite large similarities might adopt quite different backbone conformations when binding to the AT2 receptor.

AT1 antagonists constitute a new generation of drugs for the treatment of hypertension; they are designed and synthesized to mimic the C-terminal segment of angiotensin II and to block its binding action on AT1 receptor. For this reason, the conformational analysis of angiotensin II and its derivatives as well as the AT1 antagonists belonging to SARTAN's class of molecules were studied.[290] Such antagonists assist in the design of new analogs with better pharmacological profiles. Thus, nonpeptidyl (5*S*)-1-benzyl-5-(1*H*-imidazolylmethyl)-2-pyrrolidone, which mimics the His^6–Pro^7–Phe^8 part of angiotensin II, was synthesized, its conformation analyzed, and its antihypertensive activity was evaluated.

Angiotensin peptides have been demonstrated to modulate cellular proliferation, angiogenesis, and dermal repair. In a report, the effects of an analogue of the active angiotensin peptide angiotensin(1–7), namely Nle^3-angiotensin(1–7) on the healing of epithelial wounds are presented.[291] Full healing was observed for 60 percent of the diabetic mice treated with Nle^3-angiotensin(1–7). Administration of Nle^3-angiotensin(1–7) reduced fibrosis and scarring in the healing wounds. The action of this peptide was blocked by the AT receptor antagonist D-Ala^7-angiotensin(1–7), which suggests that this receptor is involved in the healing responses to exogenous Nle^3-angiotensin(1–7). These data suggest that this novel angiotensin peptide has the potential to be of benefit in accelerating wound repair and reducing scar formation.

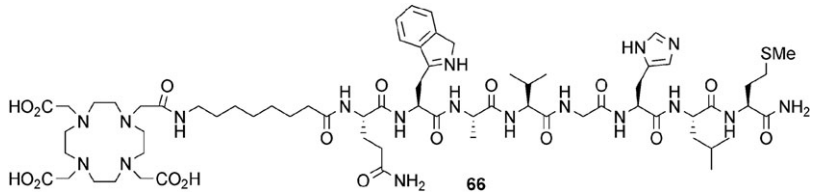
The decapeptide LVV-hemorphin-7 (LVVYPWTQRF) binds with high affinity to the angiotensin IV receptor, eliciting numerous of physiological effects, including cellular proliferation and memory enhancement. In a current study, a series of alanine-substituted and *N*- or *C*-terminally modified analogues of LVV-hemorphin-7 were evaluated for their abilities to compete for ^{125}I-angiotensin IV binding in sheep adrenal and cerebellar membranes.[292] C-Terminal deletions of LVV-hemorphin-7 resulted in modest changes in affinity, whereas deletion of the first three N-terminal residues abolished binding. Monosubstitutions of Tyr^4 and Trp^6 with alanine resulted in a 10-fold reduction in affinity. It was found that the Val^3 residue is crucial for LVV-hemorphin-7 binding, whereas the C-terminal domain seems to play a minor role.

New 1,2,4- and 1,3,4-oxadiazole derivatives as non-peptide angiotensin II receptor antagonists have been synthesized.[293]

4.5 Bombesin/neuromedin analogues

Bombesin, a 14 amino acid peptide, is an analogue of human gastrin releasing peptide that binds to GRP receptors with high affinity and specificity. The GRP receptor is overexpressed on a variety of human cancer cells including prostate, breast, lung, and pancreatic cancers. Solid-phase peptide synthesis was designed to produce a 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*′′,*N*′′′-tetraacetic acid (DOTA) conjugate with bombesin analogue (**66**).[294] The new DOTA-conjugate was metallated with ^{177}Lu(III)Cl$_3$ or non-radioactive Lu(III)Cl$_3$. The ^{177}Lu-DOTA-8-Aoc-bombesin(7–14)NH$_2$ conjugate was found to exhibit optimal pharmacokinetic properties in CF-1 normal mice. *In vitro* and *in vivo* models demonstrated the ability of the ^{177}Lu-DOTA-8-Aoc-bombesin(7–14)NH$_2$ conjugate to specifically target GRP receptors expressed on PC-3 human prostate cancer cells.

Other radiometal-labeled diethylenetriaminepentaacetic acid (DTPA) containing bombesin derivatives were synthesized and studied for competition with binding of [I-125-Tyr^4]bombesin to the GRP receptor.[295] The substitutions were the followings: DTPA-[Pro^1,Tyr^4]bombesin, DOTA-[Pro^1,Tyr^4]bombesin, DTPA-[Lys^3,Tyr^4]bombesin and DOTA-[Lys^3,Tyr^4]bombesin. The In-111-labeled bombesin analogues



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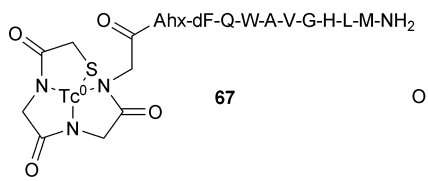
were studied *in vitro* for binding and internalization by GRP receptor-expressing CA20948 and AR42J pancreatic tumor cells as well as *in vivo* for tissue distribution in rats. In-111-DTPA-[Pro^1,Tyr^4]bombesin proved to be a promising radioligand for scintigraphy of GRP receptor-expressing tumors.

The specific aim of a study was to develop a Re-188(I)-radiolabeled BBN analogue that maintains high specificity for the GRPr *in vivo*.[296] A preselected synthetic sequence *via* solid phase peptide synthesis (SPPS) was designed to produce a Dpr-BBN (Dpr = diaminopropionic acid) conjugate with the following general structure: Dpr–X–Q–W–A–V–G–H–L–M–(NH_2), where the spacer group, X = Serylseryl-serine. Results from *in vitro* and *in vivo* models demonstrated the ability of these derivatives to target specifically GRP receptors on human, prostate, and cancerous PC-3 cells.

The same research group published Tc-99m-radiolabeled BBN analogues.[297] The conjugates had the following general structure: DMG–S–C–G–X–Q–W–A–V–G–H–L–M–(NH_2), where the spacer group, X = 0 (no spacer), ω-$NH_2(CH_2)_2COOH$, ω-$NH_2(CH_2)_4COOH$, ω-$NH_2(CH_2)_7COOH$, or ω–NH_2-$(CH_2)_{10}COOH$-N3S-BBN (N3S = dimethylglycyl-L-seryl-L-cysteinylglycinamide). *In vitro* and *in vivo* models demonstrated biological integrity of the new conjugates.

In a short review[298] numerous radiolabeled bombesin conjugates targeting bombesin 2 receptor subtype were discussed. The conjugates have shown promising diagnostic and therapeutic efficacy toward the design of site-specific radiopharmaceuticals that target cancer cells over-expressing the appropriate GRP receptor subtype.

A new chelation strategy applying bis-mercaptoacetyl functionalized diaminopropionic acid or mercaptoacetyl–Gly–Gly–Gly radiometal-chelating centers for radiolabeled bombesin derivatives was described.[299] The ^{99m}Tc-labeled peptides (**67** and **68**) were derived from a bombesin fragment containing N_3S- or N_2S_2-chelating moiety, respectively.



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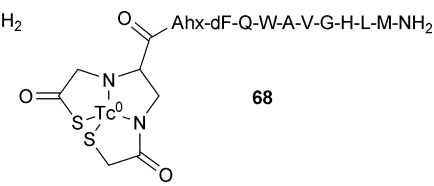

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4.6 Bradykinin analogues

Several bradykinin analogues containing numerous non-natural amino acids as indanylglycine, octahydroindole-2-carboxylic acid, cycloheptylglycine, pentafluorophenylalanine were synthesized and tested for their agonist or antagonist activity in SHP-77 cells.[300]

The B_1 and B_2 receptor activity of numerous bradykinin analogues were investigated on rat or guinea pig ileum.[301] This study shows that bradykinin B_2 receptor antagonists often have an additional blocking activity on the B_1 receptor. Analogues with replacement of proline by alkyl-substituted phenylalanine at position 7 are as effective on the B_1 receptor as the entire molecules and have a stronger antagonistic

effect than on the B_2 receptor. A corresponding desArg^9-compd. has a specific effect on the B_1 receptor and a very high antagonistic potency. [L-NMPhe^2]bradykinin as a compound without any replacement at position 7 or 8 shows antagonistic activity as well.

The synthesis and some pharmacological properties of eight new analogues of a previously synthesized bradykinin antagonist, D-Arg–Arg–Pro–Hyp–Gly–Thi–Ser–D-Phe–Thi–Arg were described.[302] Two peptides were designed by substitution of Ser^6 with L-1- and L-2-naphthylalanine. In two further analogues this modification was combined with placement in position 7 of D-naphthylalanine residue. The activity of these analogues was assessed by their ability to inhibit vasodepressor response to exogenous bradykinin (rat blood pressure test). These results indicate that the modifications proposed decreased significantly the B_2 antagonistic activity.

Spin-labeled analogues of bradykinin were synthesized containing the amino acid TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid) either before Arg^1 (TOAC0-BK) or replacing Pro^3 (TOAC3-BK).[303] Whereas the latter is inactive, the former retains about 70% of BK's activity in isolated rat uterus.

A review discussed studies that are directly relevant to the targeting of human bradykinin receptors as a therapeutic intervention and included recent data to illustrate new avenues for the therapeutic application of kinin analogues.[304]

4.7 Cholecystokinin analogues, growth hormone-releasing peptide and analogues

The carboxyl terminal octapeptide of cholecystokinin (CCK-8) has been hypothesized to account for the bioactivity of forms of cholecystokinin. However, the physiological relevance of CCK-58 has not been rigorously examined. Canine-sulfated CCK-58 was synthesized and conditions determined for its unblocking and purification that preserved the sulfated tyrosine.[305] For the synthesis, manual Fmoc strategy was chosen, the incorporation of the sulphated tyrosine was made *via* commercially available Fmoc–Tyr(SO_3H)–OH.

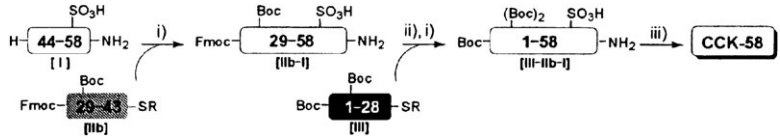
Several novel peptide ligands having opioid agonist and CCK antagonist activity for the treatments of neuropathic pain were designed and synthesized.[306] The structures of these analogues are shown in Fig. 7.

Thioester segment condensation was applied to the chemical synthesis of high molecular weight isoform of cholecystokinin.[307] For the synthesis of the partially protected segments, Fmoc-SPPS were applied. The tyrosine sulphate was incorporated as Fmoc–Tyr(SO_3Na)–OH and the C-terminal dipeptide (Fmoc–Asp–Phe–NH_2) was linked with chlorotrityl resin *via* the β-carboxyl group of Asp (Scheme 2).

A cyclic CCK-8 analogue, cyclo29,34(Dpr^{29},Lys^{34})-CCK-8 (Dpr = L-2,3-diaminopropionic acid), has been designed on the basis of the NMR structure of the bimolecular complex between the N-terminal fragment of the CCK_A receptor and its natural ligand CCK-8. The conformational features of cyclo29,34(Dpr^{29},Lys^{34})-CCK-8 have been detected by NMR spectroscopy.[308] The structure of the cyclic peptide in aqueous solution is found to be in a relaxed conformation, with the backbone and Dpr^{29} side chain atoms making a planar ring and the N-terminal tripeptide extending approximately along the plane of this ring. In DPC/water, the cyclic peptide adopts a "boat-shaped" conformation, which is more compact than that found in aqueous solution. A careful comparison of the NMR structure of the

H-Tyr-Gly-Trp-NMeNle-Asp-Phe-NH_2
H-Tyr-DAla-Gly-Trp-NMeNle-Asp-Phe-NH_2
H-Tyr-DPhe-Gly-Trp-Nle-Asp-Phe-NH_2
H-Tyr-DAla-Gly-Trp-Nle-Asp-Phe-NH_2
H-Tyr-DPhe-Gly-DTrp-NMeNle-Asp-Phe-NH_2
H-Tyr-DPhe-Gly-DTrp-Nle-Asp-Phe-NH_2

Fig. 7



Scheme 2 (i) $AgNO_3$ (3 equiv.), HOOBt (30 equiv.), DIEA (20 equiv.), DMSO, 25 °C, 24 h. (ii) 25% piperidine in DMF–DMSO, 25 °C, 3 h, then gel-filtration. (iii) 90% aq. TFA, 0 °C, 2 h.

cyclic peptide in a DPC micelle aqueous solution with the structure of the rationally designed model underlines that the turn-like conformation in the Trp^{30}–Met^{31} region is preserved, such that the Trp^{30} and Met^{31} side chains can adopt the proper spatial orientation to interact with the CCK_A receptor.

The cholecystokinin receptors CCK1R and CCK2R exert important central and peripheral functions by binding the neuropeptide cholecystokinin. Because these receptors are potential therapeutic targets, great interest has been devoted to the identification of efficient ligands that selectively activate or inhibit these receptors. A complete mapping of the CCK binding site in these receptors would help to design new CCK ligands and to optimize their properties. In this view, a molecular model of the CCK2R occupied by CCK was built to identify CCK2R residues that interact with CCK functional groups.[309] Direct interaction was demonstrated between His 207 in the CCK2R and Asp 8 of CCK. Two residues that had not been revealed in the previous mutagenesis studies, Tyr 189 (Y4.60) and Asn 358 (N6.55), were identified in interaction *via* hydrogen bonds with the C-terminal amide of CCK, a crucial functional group of the peptide. Mutagenesis of Tyr 189 (Y4.60) and Asn 358 (N6.55) as well as structure-affinity studies with modified CCK analogues validated these interactions and the involvement of both residues in the CCK binding site.

4.8 Integrin-related peptide and non-peptide analogues

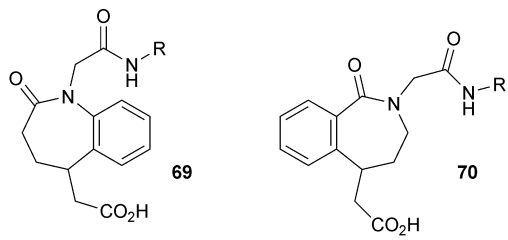
Integrins are adhesion molecules that transmit signals across the plasma membrane by undergoing structural rearrangements. From a therapeutic point of view, integrins are probably the most important class of cell-adhesion molecules. Recent progress in the development of integrin antagonists have been summarized in a 111-reference review.[310] According to this publication, small molecule integrin antagonists fall into three different classes each of which affect the equilibria that relate integrin conformational states, but in different ways. A study reviewed the discovery of small molecule antagonists to the integrins $\alpha_{IIb}\beta_3$, $\alpha_L\beta_2$, $\alpha_4\beta_1$ and $\alpha_4\beta_7$.[311]

4.8.1 IIb/IIIa antagonists. Integrin $\alpha_{IIb}\beta_3$ is the major membrane protein on the surface of platelets. Since platelet aggregation is implicated in many pathological situations such as myocardial infarction, stroke, unstable angina and coronary artery disease, potent and safe antiplatelet agents would be useful for treating and preventing these diseases. The three GPIIb/IIIa antagonists which are currently marketed can only be intravenously administered. To clearly understand how $\alpha_{IIb}\beta_3$ antagonists affect the receptor, recent advances in the investigation of the structure of integrin $\alpha_{IIb}\beta_3$, its ligand recognition, activation, and antagonism have been reviewed.[312] Another review described 16 patents on new GPIIb/IIIa antagonists and more than 40 molecules having strong activity against this integrin.[313] An article demonstrated that sCD40, a soluble $\alpha_{IIb}\beta_3$ ligand, induced platelet stimulation.[314] This outside-in signaling event appeared to be mediated by tyrosine phosphorylation of β_3. The oral administration of the glycoprotein IIb/IIIa antagonist CRL42796 prevented artery thrombosis in response to deep vessel wall injury.[315] TRA-418, a novel TP-receptor antagonist, and IP-receptor agonist, is more advantageous as an antiplatelet agent than TP-receptor antagonists or IP-receptor agonists separately

used.[316] Hookworms are a leading cause of iron deficiency anemia in the developing world. They evolved potent mechanisms of interfering with mammalian hemostasis for the purpose of facilitating bloodfeeding. Two hookworm inhibitors that block the binding of platelet GPIIb/IIIa and GPIa/IIa to their ligands have been isolated by RP-HPLC and cloned.[317]

4.8.2 $\alpha_v\beta_3$ antagonists. Since $\alpha_v\beta_3$ expression is highly restricted during tumor invasion and metastasis, the molecule represents an interesting target for diagnosis of rapidly growing solid tumors. An article reviewed radiolabeled integrin $\alpha_v\beta_3$ receptor antagonists as radiopharmaceuticals for tumor imaging.[318] Another review has focused on the key role of the α_v integrin ($\alpha_v\beta_3$ and $\alpha_v\beta_5$) in vascular disorders such as restenosis and angiogenesis-mediated disorders.[319] Various integrin antagonist candidates including antibodies, cyclic peptides, peptidomimetics, and non-peptides have also been summarized.

Efficient solid-phase synthesis and SAR of 1-substituted-4-amino-1*H*-pyrimidin-2-ones as highly potent and selective $\alpha_v\beta_3$ receptor antagonists were described.[320] The most potent compounds exhibited IC_{50} values in the nano- to subnanomolar range. Design, synthesis and SAR of 1,5- and 2,5-substituted tetrahydrobenzazepinone-based $\alpha_v\beta_3$ antagonists having the general formulas of (**69**) and (**70**) were published by the same group.[321] 7-Methoxy analogue (**71**) was identified as an antagonist displaying highest *in vitro* potency with an IC_{50} of 0.26 nM in the $\alpha_v\beta_3$ binding assay. Potent $\alpha_v\beta_3$ antagonists were identified by systematic positioning of the guanidinyl moiety around the aryl-thiophene.[322] Compound (**72**) was found as a highly potent antagonist which exhibited the best selectivity against $\alpha_{IIb}\beta_3$ of the series.



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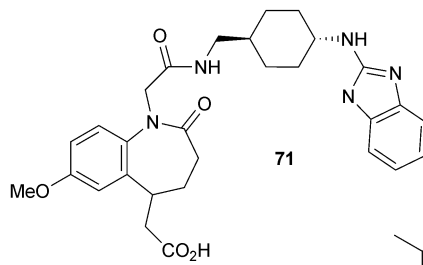

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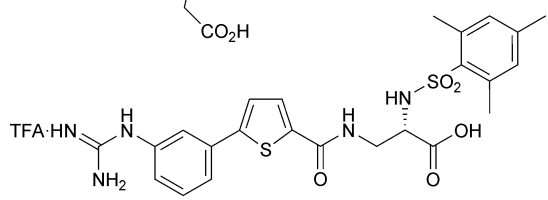

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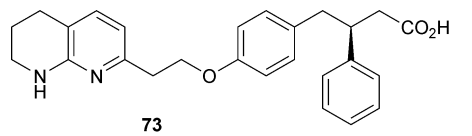
A novel class of biphenyl vitronectin receptor antagonists was identified from a focused combinatorial library based on *para*-bromo-phenylalanine.[323] Their binding mode could be rationalized by computational docking studies using the X-ray structure of $\alpha_v\beta_3$. A series of phenylbutyrates derivatives as new integrin $\alpha_v\beta_3$ antagonists were derived conceptually from opening the seven-membered ring of

SB-265123, a potent vitronectin receptor antagonist.[324] Derivative (**73**) showed good potency and excellent bioavailability (approximately 100% in rats). Two novel series of small-molecule RGD mimetics containing either a substituted pyridone (**74**) or pyrazinone (**75**) central cores were prepared.[325] All the compounds were selective against the related platelet receptor $\alpha_{IIb}\beta_3$. An efficient and stereoselective route to a new series of chain-shortened, pyrrolidinone-containing $\alpha_v\beta_3$ receptor antagonists was described, utilizing an asymmetric oxazolidinone alkylation and Friedländer condensation as key steps.[326] Compound (**76**) was identified as a potent, selective antagonist of the $\alpha_v\beta_3$ receptor.[327] On the basis of its excellent *in vitro* profile (IC_{50} = 0.08 nM) and good pharmacokinetics, this antagonist was selected for clinical development. A series of oxidized derivatives of (**76**) were prepared which proved useful in the identification of active metabolites generated by either *in vitro* or *in vivo* metabolism. Synthesis of 5-carbonyl-1,3-dihydro-1,3-dioxo-2H-isoindole-2-propanoic acid integrin antagonists was published.[328]



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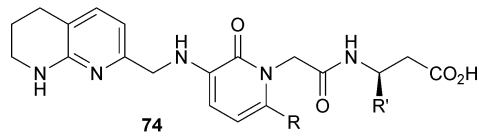

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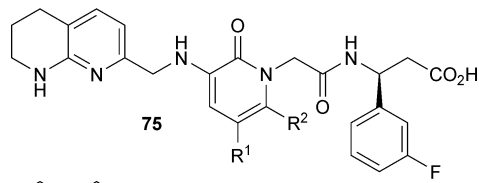

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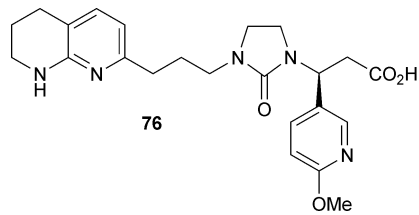

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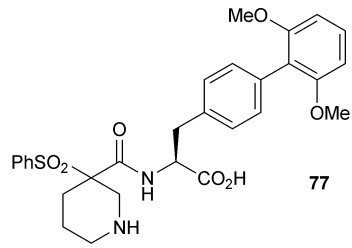
The first crystal structure of a disintegrin, trimestatin, found in snake venom, has been reported.[329] The structure at 1.7 Å resolution revealed that a number of turns and loops formed a rigid core stabilized by six disulfide bonds. Starting from the X-ray structure of the extracellular segment of the $\alpha_v\beta_3$ receptor with a cyclic RGD ligand bound to the active site, it was possible to determine the receptor-bound conformations and the mutual alignment of the most representative $\alpha_v\beta_3$ ligands.[330] A detailed analysis of the interactions between ligands and $\alpha_v\beta_3$ receptors pointed out which interaction site in the binding pocket might be responsible for the variation in biological activity and which additional interaction could be taken account for the design of new compounds. Design, synthesis, and evaluation of radiolabelled $\alpha_v\beta_3$ receptor antagonists for tumor imaging and radiotherapy have been reported.[331] TA138 was prepared by the conjugation of macrocyclic chelator DOTA to a peptidomimetic vitronectin receptor antagonist SH066. TA138 slowed tumor growth at a dose of 15 mCi/m(2), and a regression of tumors in the c-neu Oncomouse((R)) model.

4.8.3 $\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$ and $\alpha_L\beta_2$ antagonists. Most lymphocytes, except neutrophils, express very late activating antigen-4 (VLA-4) on their surface and they interact with endothelial vascular cell adhesion molecule-1 (VCAM-1). This interaction can be disrupted by VLA-4 antagonists. A 151-reference review provided an update of VLA-4 antagonists that had appeared since 2001.[332] Four classes of VLA-4 antagonists were discussed: (i) cyclic peptides, (ii) linear peptides (LDV mimetics), (iii) *N*-acylphenylalanine derivatives, and (iv) unique structures. Clinical data for natalizumab, a rationally designed monoclonal antibody to human α_4 integrin, which was shown as a promising agent in phase II trials in patients with Crohn's disease and multiple sclerosis, were summarized in another review.[333] *N*-(3-Phenylsulfonyl-3-piperidinoyl)-(L)-4-(2,6′-dimethoxyphenyl)phenylalanine (**77**), a potent and selective VLA-4 antagonist, having the IC_{50} of 90 pM, was identified from the SAR of 1-sulfonyl-cyclopentyl carboxylic acid amides.[334] It was demonstrated that isonicotinoyl-(L)-aminophenylalanine derivatives were potent VLA-4 antagonists which possessed slow off-rate.[335] One of the compounds (**78**) showed activity in reducing eosinophil trafficking in an OVA asthma model.



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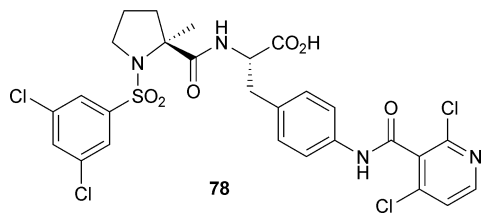
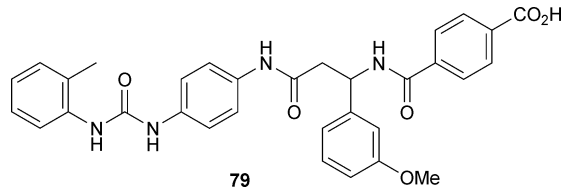


78

A ^{35}S-labeled compound was used as a model ligand to study the effect of divalent cations on the activation state and ligand binding properties of α_4 integrins.[336] The study demonstrated that $\alpha_4\beta_1$ and $\alpha_4\beta_7$ have distinct binding properties for the same ligand. A series of dehydrophenylalanine derivatives has been described where the *Z* isomers are potent VLA-4 antagonists but are subject to rapid clearance and the *E* isomers have poor activity but a slower rate of clearence.[337] These constrained molecules led to the discovery of a novel class of benzodiazepine VLA-4 antagonists. A novel class of $\alpha_4\beta_1$ integrin antagonists derived from terephthalic acid as exemplified by (**79**) was described.[338] The most potent antagonists inhibited the adhesion in a cell based assay in the low and sub micromolar range. Predictive CoMFA and CoMSIA 3-D-QSAR models were developed for tetrahydrofuroyl-L-phenylalanine derivatives as VLA-4 antagonists.[339] Based on the spatial arrangement of the various field contributions, novel molecules with improved activity can be designed. A synthetic antagonist of VLA-4, TBC 3486, effectively delayed and reduced the acute phase of experimental autoimmune encephalomyelitis, a model of multiple sclerosis.[340]

A novel, short $\alpha_1\beta_1$ antagonist, obtustatin, isolated from the venom of *Vipera lebetina obtusa* was described. Amino acid sequence, homology-modeling[341] as well as NMR solution structure of this 41-amino acid non-RGD disintegrin were also published.[342] The venom of the snake *Echis multisquamatus* was the source of EMS16, a glycoprotein Ia/IIa (integrin $\alpha_2\beta_1$) antagonist. Purification, sequence[343] and structural characterization[344] were presented. The azabicyclo[5.2.0]nonan-2-one



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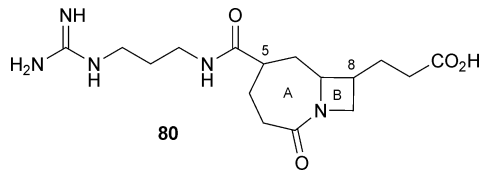
lactam (**80**) adequately substituted on both cycles A and B mimics the conformationally constrained β-turn of the tripeptide RGD signaling motif of fibronectin and serves as a novel class of $\alpha_5\beta_1$ antagonist.[345] A novel mechanistic class of $\alpha_L\beta_2$ integrin antagonists that bind to the metal ion-dependent adhesion site of the β_2 I-like domain and prevent its interaction with and activation of the α_L I domain has been described.[346]



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4.9 GnRH analogues

Photodynamic therapy uses a combination of light, oxygen, and a photosensitizer to induce the death of malignant cells. To improve the selectivity of a photosensitizer toward cancerous cells that express gonadotropin-releasing hormone (GnRH) receptors, protoporphyrin IX was conjugated to a GnRH agonist, [D-Lys^6]GnRH, or to a GnRH antagonist, [D-Glp^1, D-Phe^2, D-Trp^3, D-Lys^6]GnRH.[347] Although these conjugates had lower binding affinity to rat pituitary GnRH receptors than their parent analogues, they fully preserved their agonistic or antagonistic activity *in vitro* and *in vivo*. The GnRH agonist conjugate proved to be long-acting *in vivo*. The conjugates, notably the agonist, were more phototoxic toward pituitary gonadotrope αT3-1 cell line than was unconjugated protoporphyrin IX. The selectivity of the GnRH antagonist conjugate to gonadotrope cells in a primary pituitary culture was approximately 10 times higher than that of the unconjugated protoporphyrin IX. Thus, GnRH-based conjugates may affect cancer cells not only by acting as classic GnRH analogues to reduce the plasma levels of steroids by desensitization of the pituitary gland but also by selective photodamage of cells that express GnRH receptors.

Recently three types of non-mammalian gonadotropin-releasing hormone receptors (GnRHR) in the bullfrog and a mammalian type-II GnRHR in green monkey cell lines were identified. All these receptors responded better to GnRH-II than GnRH-I, while mammalian type-I GnRHR showed greater sensitivity to GnRH-I than GnRH-II.[348] Ligand–receptor binding assays revealed that [D-Ala^6]GnRH-II and Triptorelin have higher affinities for non-mammalian GnRHRs but lower affinities for mammalian type-I GnRHR than GnRH-II and Cetrorelix, respectively.

Approximately 80% of human ovarian and endometrial cancers and 50% of breast cancers express GnRH and its receptor as part of an autocrine regulatory system. After binding of its ligand the tumor GnRH receptor couples to G-protein αi and activates a variety of intracellular signaling mechanisms. Recently, a second type of GnRH receptor, specific for GnRH-II, has been identified in ovarian and endometrial cancers, which transmits significantly stronger antiproliferative effects than the GnRH-I receptor. In animal models of human cancers, GnRH antagonists had stronger antitumor effects than GnRH agonists. Based on this, cytotoxic GnRH analogues have been developed, where for example doxorubicin was covalently coupled to GnRH analogues.[349] These compounds have superior antitumor effects

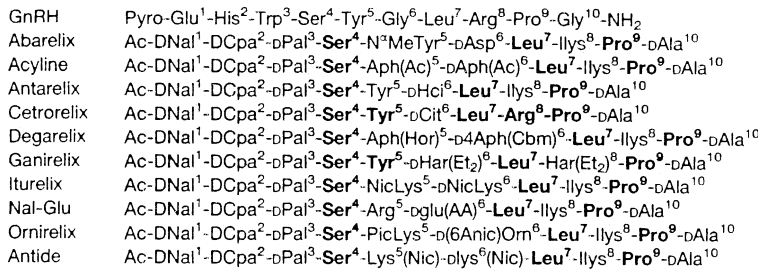


Fig. 8

in cancers expressing GnRH receptors as compared with native doxorubicin and allow for a targeted cytotoxic chemotherapy of gynecological and breast cancers.

In a review, the pharmacological application of numerous antagonistic analogues the decapeptide GnRH were discussed.[350] The chemical structures of the investigated antagonists are summarized in Fig. 8. Many of them are in clinical trial in the treatment of cancer or regulation of the reproductive system.

4.10 MHC class I and II analogues

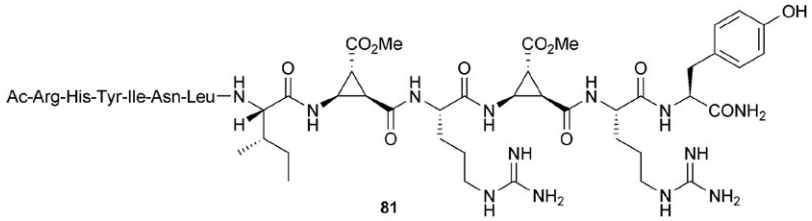
A previously reported 16-amino acid peptide analogue, AEGFSYTVANKAKGIT, derived from pigeon cytochrome c (p43–58) by a two-residue substitution, could bind broad ranges of MHC Class II types and activate helper T cells in mice. A present study showed that it was possible to enhance the antitumor effects elicited by vaccine immunotherapy by using this peptide analogue.[351] The use of DNA encoding PAN-IA peptides seemed to be more suitable for DNA-based vaccines than the use of Pan-IA peptides. Retro-inverso (ri) analogues of model T and B cell epitopes were designed and assayed in order to understand the basis of functional ri-antigenic peptide mimicry.[352] While the T cell epitopes to MHC I could be mimicked by a ri analogue, the highly specific binding of a hepatitis B virus protein, PS1, through a β-turn conformation could not be mimicked by a similar analogue. Identifying five new epitopes, a study extended to eight the number of known melanoma-associated antigen epitopes shared by the majority of HLA-B*3501 melanoma tumors.[353] The observations confirmed the ability of certain antigenic peptides to bind to more than one MHC class I gene products. Sixteen novel peptide sequences associated with the HLA-A2.1 molecule expressed in two ovarian cancer cell lines previously exposed to IFN-γ have been identified.[354] All 16 peptides are homologous to known proteins in the database.

4.11 Neuropeptide Y (NPY) analogues

The conformation of several neuropeptide Y antagonists (Ile–Asn–Pro–Ile–Tyr–Arg–Leu–Arg–Tyr–OMe and Ile–Asn–Pro–Ile–Tyr–Asn–Leu–Arg–Tyr–NH_2) were investigated by NMR and CD spektroscopy.[355] In contrast of the very slight structural differences, the above methods and molecular dynamics simulations showed significant changing in the 3D structure, which may be responsible for the Y_1 selectivity of the above peptides.

Cyclic beta amino acid containing analogues of NPY fragments were synthesized by Fmoc/*tert*-butyl solid-phase protocol and tested for their $Y_1/Y_2/Y_5$ receptor selectivity.[356] One of the most rigidified peptides (**81**) was a C-terminal NPY analogue containing two β-aminocyclopropane carboxylic acid residues.

The 69 amino acid residue long prohormone of the NPY was prepared by combination of chemical synthesis, protein expression in *E. coli* and thioester ligation.[357] The described method (expressed protein ligation) allows the production of Pro-Neuropeptide Y with modified primary structures.



D-Trp[34] analogue of NPY was investigated for their action in Y_5 receptor mediated obesity.[358] The results suggested the key role of Y_5 receptor in regulating the energy homeostasis.

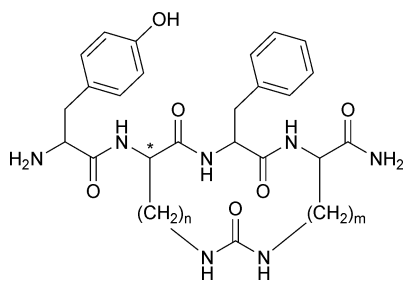
Several novel modified and radiolabelled neuropeptide Y analogues were developed.[359] Depending on the radioisotope the resulting radiolabelled NPY analogues can be used for diagnostic or therapeutic applications and enable the investigation of single receptor subtypes *in vitro* and *in vivo*.

4.12 Opioid (neuropeptide FF, enkephalin, nociceptin, deltorphin and dynorphin) peptides

A new family of cyclic opioid peptide analogs (Fig. 9) related to the 1–4 sequence of dermorphin/deltorphin (Tyr–D-Aaa^2–Phe–Aaa^4–NH_2) has been synthesized.[360] The synthesis of the linear precursor peptides was accomplished by the solid-phase method and ring formation was achieved *via* a ureido group incorporating the side chain amino functions of D-Aaa^2 (D-Lys, D-Orn) and Aaa^4 (Lys, Orn, Dab, Dap). The peptides were tested in the guinea-pig ileum (GPI) and mouse vas deferens (MVD) assays. Most of them showed high agonist potency in the GPI assay. The peptide containing D-Lys in position 2 and Dab in position 4 was 210 times more active than enkephalin.

Analogues of D-Ala^{2}deltorphin I (H–Tyr–D-Ala–Phe–Asp–Val–Val–Gly–OH), where Asp^4 is replaced by other amino acids, were synthesized by Fmoc solid-phase methodology.[361] For example, H–Tyr–D-Ala–Phe–Xaa–Val–Val–Gly–NH_2 [Xaa = Asn, N^4-methoxy-L-2,4-diaminobutanoic acid, Asn[β-D-GlcNAc(Ac3)], Asn(β–D-GlcNAc), N^4-methoxy, N^4-(β-D-glucosyl)-L-2,4-diaminobutanoic acid] were synthesized. *In vitro* biological activity of the peptides was compared with that of the μ-opioid receptor agonist dermorphin in guinea pig ileum (GPI) preparations and with that of the δ-opioid receptor agonist deltorphin I in mouse vas deferens (MVD) preparations. The substitution of Asp^4 with Asn failed to affect drastically the K_i and IC_{50} values for δ-sites, suggesting that an electrostatic interaction does not play an essential role in the binding to δ-opioid sites. The steric hindrance of the side chain of the residue in position 4 affects binding to δ-sites.

To improve the oral bioavailability of a dermorphin tetrapeptide analogue, N^{α}-1-iminoethyl–Tyr–D-MetO–Phe–MeβAla–OH, which has a potent analgesic activity



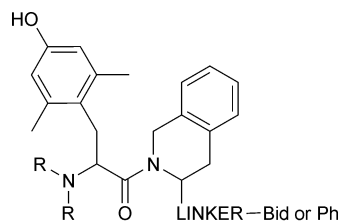
Lys^2/Lys^4	n = 4, m = 4
Lys^2/Orn^4	n = 4, m = 3
Lys^2/Dab^4	n = 4, m = 2
Lys^2/Dap^4	n = 4, m = 1
Orn^2/Lys^4	n = 3, m = 4
Orn^2/Orn^4	n = 3, m = 3
Orn^2/Dab^4	n = 3, m = 2
Orn^2/Dap^4	n = 3, m = 1

Fig. 9

after oral administration,[362] various derivatives were synthesized to increase lipophilicity by esterification of the C-terminal carboxyl group and/or acylation of the phenolic hydroxyl group on Tyr^1. Antinociceptive activity was evaluated after s.c. or oral administration using the mouse tail pressure test. As a result, increased antinociceptive activity after oral administration as well as an improved ED_{50}(p.o.)/ED_{50}(s.c.) ratio, which is an indicator of oral bioavailability, were found for some compounds. With regard to the improvement of bioavailability, derivatives with acylation of the phenolic hydroxyl group on Tyr^1 showed better results than peptides esterified on the C-terminal carboxyl group.

To investigate the effectiveness of a 2′,6′-dimethylphenylalanine (Dmp) residue as an aromatic amino acid surrogate, endomorphin 2 (EM2: Tyr–Pro–Phe–Phe–NH_2) analogues were prepared in which the constitutive aromatic amino acids (Tyr^1, Phe^3, or Phe^4) were replaced by Dmp or its isomer, D-Dmp. Replacement of Phe^3 by Dmp increased the affinity over 10-fold for both μ- and δ-opioid receptors, without affecting receptor selectivity.[363] In contrast, replacement of Phe^4 considerably reduced the μ-receptor affinity and selectivity. These data indicated that the Dmp-substitution of Phe^3, but not Phe^4, in EM2 is favorable for improving μ-receptor specificity. Inversion of the chirality of the substituted Dmp residue resulted in marked decrease in the μ-receptor affinity. Replacement of Tyr^1 by Dmp yielded an analogue that exhibited only a limited decrease in μ-receptor affinity and GPI potency, despite the lack of a phenolic hydroxyl group at the N-terminal residue. In contrast, D-Dmp^1- or Phe^1-substitution of Tyr^1 resulted in a significant decrease in μ-receptor affinity and GPI potency. These results suggested that the Dmp residue can mimic Tyr^1, which is one of the critical structural elements of opioid peptides.

N,*N*-Dimethylation of the H–Dmt–Tic–NH–CH(R)–R′ series of compounds having the general formula of (**82**) produced no significant affect on the high δ-opioid receptor affinity (K(i) = 0.035–0.454 nM), but dramatically decreased that for the μ-opioid receptor.[364] The effect of *N*-methylation was independent of the length of the linker (R); however, the bioactivities were affected by the chemical composition of the third aromatic group (R′): phenyl (Ph) (5′–8′) elicited a greater reduction in m-affinity (40–70-fold) compared to analogues containing 1*H*-benzimidazole-2-yl (9-fold). The major consequences of *N*,*N*-dimethylation on *in vitro* bioactivity were: loss of δ-agonism coupled with the appearance of potent δ-antagonism and consistent loss of μ-affinity resulted in enhanced δ-opioid receptor selectivity.



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New dermorphin and [D-Ala^2]deltorphin I analogues in which the phenylalanine, the tyrosine or the valine residues have been substituted by the corresponding *N*-alkylglycine residues were described (Fig. 10).[365] Structural investigations by CD measurements in different solvents and preliminary pharmacological experiments were carried out on the resulting peptide–peptoid hybrids. The contribution from aromatic side chain residues is prominent in the CD spectra of dermorphin analogues and the assignment of a prevailing secondary structure could be questionable. In the CD spectra of deltorphin analogues the aromatic contribution is lower and the dichroic curves indicate the predominance of random conformer populations. The disappearance of the aromatic contribution in the [$Ntyr^1$,D-Ala^2]–deltorphin spectrum could be explained in terms of high conformational freedom of the N-terminal residue. The binding to opioid receptors was tested on crude

H-Ntyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2	[$Ntyr^1$]-dermorphin
H-Tyr-D-Ala-Nphe-Gly-Tyr-Pro-Ser-NH_2	[$Nphe^3$]-dermorphin
H-Ntyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2	[$Ntyr^1$,D-Ala^2]-deltorphin I
H-Tyr-D-Ala-Nphe-Asp-Val-Val-Gly-NH_2	[D-Ala^2,$Nphe^3$]-deltorphin I
H-Tyr-D-Ala-Phe-Asp-Nval-Val-Gly-NH_2	[D-Ala^2,$Nval^5$]-deltorphin I
H-Tyr-D-Ala-Phe-Asp-Val-Nval-Gly-NH_2	[D-Ala^2,$Nval^6$]-deltorphin I
H-Tyr-D-Ala-Phe-Asp-Nval-Nval-Gly-NH_2	[D-Ala^2,$Nval^5$,$Nval^6$]-deltorphin I

Fig. 10

membrane preparations from CHO cells stably transfected with the μ- and δ-opioid receptors. The biological potency of peptoids was compared with that of dermorphin in GPI preparations and with that of deltorphin I in MVD preparations. All the substitutions produced a dramatic decrease in the affinity of the peptide–peptoid hybrids for both the μ- and δ-opioid receptors. $Nval^5$ and/or $Nval^6$ containing hybrids behaved as μ-opioid receptor agonists and elicit a dose-dependent analgesia (tail-flick test) when injected i.c.v. in rats.

A series of analogues of nociceptin, Noc(1–13)NH_2 (an agonist at the ORL1 receptor) was synthesized with following modifications: (i) *N*-terminal extension with Arg^0; (ii) replacement of Gly^3 by basic or polar amino acids–Arg, Asn, Lys(For) or deletion; (iii) exchange of Phe^1 or Phe^4 by Phe(NO_2); (iv) substitution of Ser^{10} with D-Ser, Pro, D-Pro. These analogues were synthesized by solid-phase methodology using Fmoc-amino acid pentafluorophenyl esters.[366] The affinity for the ORL1 and for the κ-, μ- and δ-opioid receptors was investigated by radioligand binding assay and bioactivity by a mouse vas deferens (MVD) assay. The addition of the amino acid residue Arg to the N-terminal enhances the opioid receptor affinity of Noc(1–13)NH_2 while retaining ORL1 receptor affinity at a moderate level. The replacement of Gly in position 3 by the basic or polar amino acids–Arg, Asn, Lys(For) or its deletion led to inactive analogues. The replacement of Ser in position 10 by its D-isomer, Pro and D-Pro resulted in a series of analogues with the following order of activity: Ser^{10} > D-Ser^{10} > Pro^{10} > D-Pro^{10}.

The synthesis and the pharmacological activity of a series of *N*-terminally modified hexapeptides with high affinity for ORL1 was described.[367] These compounds were tested for binding affinity using [^{3}H]Noc/Orphanin binding to human ORL1 in CHO cells, and functional activity by measuring stimulation of [^{35}S]GTPgammaS binding in CHO cell membranes. The N-terminal modifications have produced compounds that maintained very high receptor affinity, but led to significant changes in intrinsic activity. One compound, pentanoyl–RYYRWR–NH_2, with barely measurable agonist activity was tested *in vivo*. It was found to possess modest analgesic activity, but it was unable to block the morphine modulatory activity of Noc/Orphanin.

For characterization of the highly potent dermorphin-derived opioid peptide (H–Dmt–D-Arg–Phe–Lys–NH_2; Dmt, 2′,6′-dimethyltyrosine, DALDA), [^{3}H][Dmt^1]-DALDA has been synthesized *via* incorporation of 2,6-dimethyl-3,5-diiodotyrosine and its binding profile studied.[368]

Opioid peptide analogues containing either 3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid (Dhp) or (2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid [(2*S*)-Mdp] in place of Tyr^1 were synthesized.[369] Using this approach, δ-, κ- and μ-selective opioid peptide agonist peptides were successfully converted into corresponding δ-, κ- and μ-selective antagonists, whereby receptor selectivity was often maintained or even improved.

Dermorphin, and a new analogue of it, [D-Pro^6]dermorphin, were synthesized by fragment condensation in the liquid phase.[370]

The synthesis and pharmacological activity of novel nociceptin/orphanin analogues modified in the Phe^1–Gly^2 peptide bond are reported.[371] The aim of this work

was to elucidate the importance of this peptide bond for the nociceptin/orphanin receptor interaction. This study indicates that the first peptide bond in nociceptin/orphanin is important but not crucial for interaction with the nociceptin/orphanin receptor; for instance, substitution with a methyleneoxy bond generates an agonist derivative just 3-fold less potent than the reference compound.

Dermorphin and Lys^7 dermorphin, selective μ-opioid receptor ligands originating from amphibian skin, have been modified with various electrophiles in either the 'message' or 'address' sequences as potential peptide-based affinity labels for μ-receptors.[372] Introduction of the electrophilic isothiocyanate and bromoacetamide groups on the para position of Phe^3 and Phe^5 was accomplished by incorporating Fmoc–Phe(p-NHAlloc) into the peptide followed by selective deprotection and modification. The pure peptides were evaluated in radioligand binding experiments using CHO cells expressing μ- and δ-opioid receptors. In dermorphin, introduction of the electrophilic groups in the 'message' domain lowered the binding affinity by >1000-fold; only [Phe(p-NH_2)^3]dermorphin retained nanomolar affinity for μ-receptors. Modifications in the 'address' region of both dermorphin and Lys^{7}dermorphin were relatively well tolerated.

4.13 Somatostatin analogues

Recently it was reported that the somatostatin analogue TT-232, D-Phe–c(Cys–Tyr–D-Trp–Lys–Cys)–Thr–NH_2, exhibited a highly potent antitumor activity *in vitro* and *in vivo*. Instead of the disulfide bond, pyrazinone rings and aliphatic amino acids were used, which were coupled to Tyr–D-Trp–Lys, the sequence essential for antitumor activities.[373] These analogues exhibited strong antiproliferative activities. However, they did not exhibit a clear structure–activity relationship.

A new strategy was described for the synthesis of peptides having C-terminal cysteine residue, like many naturally occurring peptide acids, *e.g.*, somatostatins, conotoxins, and defensins. The synthetic problems which are documented for several current strategies can be circumvented by the new anchoring strategy, which features the following: (a) conversion of the eventual C-terminal cysteine residue, with Fmoc for N^{α}-amino protection and *tert*-Bu for C^{α}-carboxyl protection, to a corresponding *S*-xanthenyl preformed handle derivative; and (b) attachment of the resultant preformed handle to amino-containing supports. Implementation of this strategy is documented with syntheses of three small model peptides, as well as the tetradecapeptide somatostatin. Anchoring occurs without racemization, and the absence of 3-(1-piperidinyl)alanine formation is inferred by retention of chains on the support throughout the cycles of Fmoc chemistry. Fully deprotected peptides, including free sulfhydryl peptides, are released from the support in excellent yield.[374]

Hypothesizing that structural constraints in somatostatin (SRIF) analogues may result in receptor selectivity, and aiming to characterize the bioactive conformation of somatostatin at each of its five receptors, an *N*β-methylated aminoglycine (Agl) scan of the octapeptide H–c[Cys^3–Phe^6–Phe^7–D-Trp^8–Lys^9–Thr^{10}–Phe^{11}–Cys^{14}]–OH was carried out.[375] It was found that H–c[Cys–LAgl(*N*βMe,benzoyl)–Phe–D-Trp–Lys–Thr–Phe–Cys]–OH, H–c[Cys–Phe–LAgl(*N*βMe,benzoyl)–Trp–Lys–Thr–Phe–Cys]–OH, H–c[Cys–Phe–LAgl(*N*βMe,benzoyl)–D-Trp–Lys–Thr–Phe–Cys]–OH, and H–c[D-Cys–Phe–LAgl(*N*βMe,benzoyl)–D-Trp–Lys–Thr–Phe–Cys]–OH had high affinity and selectivity for sst4 (>50-fold over the other receptors). The L-configuration at positions 7 and 8 (L7, L8) yields greater sst4 selectivity than the L7, D8 configuration. Peptides with the D7, L8 (7) and D7, D8 (9) configurations are significantly less potent at all receptors. All Agl-containing analogues were first synthesized using unresolved Fmoc–Agl(*N*βMe,Boc)–OH, and the diastereomers were separated using HPLC. Chiral assignment at the Agl-containing residue was subsequently done using enzymic degradation. The results suggested that the orientation of side chains at position 6, 7, or 11 with respect to the side chains of residues 8 and 9 may be independently responsible for sst4 selectivity.

A family of high-affinity, sst4-selective cyclic somatostatin octapeptides resulted from the substitution of D-Trp[8] in H–c[Cys[3]–Phe[6]–Phe[7]–D-Trp[8]–Lys[9]–Thr[10]–Phe[11]–Cys[14]]–OH by one of the four conformationally biased stereoisomers of β-methyl-3-(2-naphthyl)alanine (β-Me2Nal) was described.[376] Whereas H–c[Cys–Phe–Phe–D-Nal–Lys–Thr–Phe–Cys]–OH has high affinity and marginal selectivity for human sst3, H–c[Cys–Phe–Tyr–D-threo–β-Me2Nal–Lys–Thr–Phe–Cys]–OH has high affinity for all sst's except for sst1; H–c[Cys–Phe–Tyr–L-threo–β-Me2Nal–Lys–Thr–Phe–Cys]–OH has high affinity for sst4, with more than 50-fold selectivity toward the other receptors. Analogues containing D- and L-erythro–β-Me2Nal instead of the corresponding threo derivatives at position 8, are essentially inactive at all receptors.

It was found that H–c[Cys–Phe–Phe–Trp–Lys–Thr–Phe–Cys]–OH, H–c[Cys–Phe–Phe–L-threo–β-MeTrp–Lys–Thr–Phe–Cys]–OH and H–c[Cys–Phe–Phe–D-threo–β–MeTrp–Lys–Thr–Phe–Cys]–OH have very high affinity for sst4 (IC_{50} = 0.7, 1.8, and 4.0 nM, respectively) and 5- to 10-fold selectivity *vs.* the other sst's. From earlier work, it was concluded that an L–amino acid at position 8 and a tyrosine or 4-aminophenylalanine substitution at position 7 may lead to high sst4 selectivity. Carbamoylation of the N-terminus of most of these analogues resulted in slightly improved affinity, selectivity, or both.[377]

Earlier studies have shown that modification of the octapeptide octreotide in positions 3 and 8 may result in compounds with increased somatostatin receptor affinity that, if radiolabeled, display improved uptake in somatostatin receptor-positive tumors. The aim of a recent research study was to employ the parallel peptide synthesis approach by further exchanging the amino acid in position 3 of octreotide and coupling the macrocyclic chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) to these peptides for labeling with radiometals like gallium-67 or -68, indium-111, yttrium-90 and lutetium-177.[378] The purpose was to find radiopeptides with an improved somatostatin receptor binding profile in order to extend the spectrum of targeted tumors. The first peptide, [^{111}In,^{90}Y-DOTA]-1-Nal[3]-octreotide (^{111}In,^{90}Y-DOTA-NOC), was isolated which showed an improved profile. The promising preclinical data justify the use of this new radiopeptide for imaging and potentially internal radiotherapy studies in patients.

[^{99m}Tc–EDDA–HYNIC–D-Phe[1],Tyr[3]]octreotide is a promising new agent with the potential to replace [^{111}In–DTPA–D-Phe[1]]–octreotide in somatostatin receptor scintigraphy.[379] This hydrazinonicotinic (HYNIC) acid derivatized somatostatin complex contains ethylenediamine-*N*,*N*′-diacetic acid (EDDA) as a coligand resulting in a high *in vitro* and *in vivo* stability.

The γ-dipeptide derivatives (Fig. 11) were prepared from alanine and lysine *via* lactams ring opening, esterification and coupling, and their conformation was

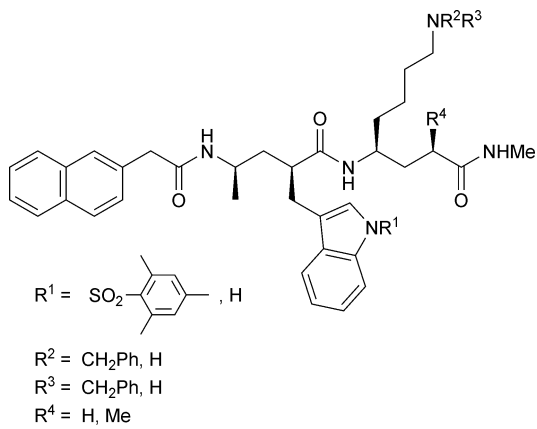


Fig. 11

studied by NMR and CD spectra.[380] The biological activities of prepared naphthylacetyl dipeptide amide derivatives were tested for their binding affinities for somatostatin receptors. The results presented in this paper promise a potential of γ-peptides for the development of peptidomimetic drugs.

Somatostatin analogues promising for peptide receptor scintigraphy (PRS) and peptide receptor radionuclide therapy (PRRT) are D-Phe–c(Cys–Tyr–D-Trp–Lys–Thr–Cys)–Thr(ol) (Tyr^3–octreotide) and D-Phe–c(Cys–Tyr–D-Trp–Lys–Thr–Cys)–Thr (Tyr3–octreotate).[381] For radiotherapeutic applications these peptides are labeled with the β-particle emitters ^{177}Lu or ^{90}Y. The therapeutic effects of these analogues chelated with DOTA were evaluated. ^{177}Lu–octreotate could reduce tumor growth to 100% cell kill and effects were dependent on radiation dose, incubation time, and specific activity used. In conclusion, Tyr^3–octreotate labeled with ^{177}Lu or ^{90}Y is the most promising analog for PRRT.

The accumulation of radioactive somatostatin analogue [^{111}In]pentetreotide in non-small cell lung cancer (non-SCLC) during scintigraphy of patients provides a rationale for investigating the efficacy of somatostatin receptor-based chemotherapy in non-SCLC.[382] Consequently, in this study, the evaluation of the antitumor effects of cytotoxic somatostatin analogue AN-238 on H838 human non-SCLC xenografted into nude mice in comparison with its cytotoxic radical, 2-pyrrolinodoxorubicin (AN-201) was made. The results indicate that patients with inoperable non-SCLC may benefit from chemotherapy targeted to somatostatin receptors based on AN-238.

Somatostatin receptors type 2 (sst2) are expressed in high concentration on numerous neuroendocrine tumors. The successful use of radiolabeled somatostatin analogues in imaging promoted further studies in utilizing them in radiopeptide therapy.[383] The somatostatin analogue [^{90}Y–DOTA–D-Phe^1–Tyr^3]octreotide (DOTATOC) possesses favorable characteristic for its therapeutic use; shows high affinity for sst2, moderately high affinity for sst5, and intermediate affinity for sst3; high hydrophilicity; stable and facile labeling with ^{III}In and ^{90}Y.

After the discovery of several specific peptide receptors in a variety of cancer types more than 10 year ago, radiolabeled peptide analogues with adequate stability, receptor binding properties, and biokinetic behavior were introduced for imaging of neuroendocrine tumors, several adenocarcinomas, lymphoma, and melanoma.[384] Although initially ^{123}I or ^{111}In were used for labeling, recent efforts have also concerned on ^{99m}Tc or PET-radionuclides (^{18}F, ^{68}Ga), as they result in better image resolution with lower radiation dose to patients. Scintigraphy with labeled somatostatin analogues (^{99m}Tc,^{111}In,^{18}F,^{68}Ga), with ^{123}I-labeled vasoactive intestinal peptide, and recently ^{99m}Tc-bombesin/GRP-or-^{111}In gastrin analogues, have shown a mean sensitivity of greater than 85% to localize deposits of tumors, with appropriate receptor expression frequently scarcely visible with other imaging procedures. Moreover, these observations introduced peptide-targeted metabolic radiotherapy for metastatic cancers.

The short-range radiation from ^{111}In (Auger-electrons) enhanced radiobiological effect, when delivered by somatostatin analogues after internalization into H69 cells. This enhanced effect of internalized ^{111}In was reduced due to increased cross-irradiation from the emitted conversion electrons.[385] The high abundance of photons significantly reduced the possibilities of using ^{111}In for therapy of somatostatin receptor-positive tumors in man.

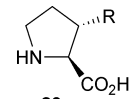
4.14 Substance P analogues

Photoreactive analogues of substance P (biotin sulfone-spacer (amino pentanoic or Gly_3)–Arg–Pro–Lys–Pro–(pBzl)Phe–Gln–Phe–Phe–Gly–Leu–Met(O_2)NH_2) with or without isotope (deuterium) labeling have been synthesized.[386]

G-Protein-coupled bombesin receptors are capable of signaling through the Gi protein even when receptor-coupling to Gq protein is blocked by [D-Arg^1,D-Phe^5,

D-Trp[7,9],Leu[11]]substance P (SpD), a neurokinin-1 receptor antagonist and "biased" agonist to bombesin receptors.[387]

Characterization of the bioactive conformation of the C-terminal tripeptide Gly–Leu–Met–NH_2 of substance P using [*trans*-3-prolinoleucine[10]]SP (**83**) and [*cis*-3-prolinoleucine[10]]SP (**84**) analogues was described.[388]



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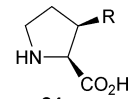

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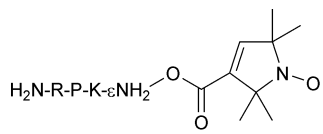
Two non-stoichiometric binding sites were characterized for the NK-1 receptor using two different types of radiolabeled analogues of substance P.[389]

The photoaffinity labeling of the SP receptor with a peptide antagonist analogue, Bapa[0][(pBzl)Phe[8],D-Pro[9],MePhe[10],Trp(CHO)[11]]SP was described.[390] The investigations using this photoreactive peptide antagonist and mass spectrometry revealed that the Met[174] side-chain within the receptor second extra cellular loop is covalently linked to the photoreactive peptide.

[Arg[6],D-Trp[7,9],NmePhe[8]]-substance P(6–11) was further investigated as a novel anticancer agent that has recently completed phase I clinical trials.[391]

Substance P (SP) was used as a model peptide to analyze the structural and biological consequences of the substitution of Phe[7] and Phe[8] by (*R*)-β2-HPhe and of Gly[9] by HGly (*R*)-β2-HAla or (*S*)-β3-HAla[7]. [(*R*)-β2-HAla[9]]SP has pharmacological potency similar to that of SP while [HGly[9]]SP and [(*S*)-β3-HAla[9]]SP show a 30- to 50-fold decrease in biological activities. The three analogues modified at position 9 are more resistant to degradation by angiotensin converting enzyme than SP and [Ala[9]]SP. NMR analysis of these SP analogues suggest that a β-amino acid insertion in position 9 does not affect the overall backbone conformation. Altogether these data suggest that [HGly[9]]SP, [(*S*)-β3-HAla[9]]SP and [(*R*)-β2-HAla[9]]SP could adopt backbone conformations similar to that of SP, [Ala[9]]SP and [Pro[9]]SP. In contrast, incorporation of β2-HPhe in position 7 and 8 of SP led to peptides that are almost devoid of biological activity. Thus, a β-amino acid could replace an β-amino acid within the sequence of a bioactive peptide provided that the additional methylene group does not cause steric hindrance and does not confine orientations of the side chain to regions of space different from those permitted in the β-amino acid.[392]

The synthesis of a spin labeled Substance P analogue (85) was described and used for electron paramagnetic resonance spectroscopy studies.[393]



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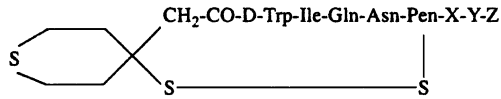
4.15 Vasopressin and oxytocin analogues

The solid-phase synthesis and some pharmacological properties of seventeen new oxytocin analogues were reported.[394] Basic modification at positions 8 and/or 9 (introduction of L-α-*t*-butylglycine [Gly(*t*Bu)]) was combined with D-Cys[6], D-Tyr-(Et)[2], Mpa[1] or Pen[1] modifications and their various combinations. These analogues were tested for rat uterotonic activity *in vitro*, in the rat pressor assay and for binding affinity to human OTR.

Fluorescently labeled analogues of oxytocin, vasopressin and desmopressin (dDAVP) fragments were prepared.[395] Hexapeptides with ornithine instead of cysteine in position 6 of the peptide chain were synthesized on solid-phase and fluorescently labeled by 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD).



Analogue	X	Y	Z
PA	Pro	Arg	Gly-NH_2
1	Pro	Arg	Gly-OH
2	Pro	Arg-NH_2	-----
3	Pro	Arg-OH	-----
4	Pro-NH_2	-----	-----
5	Pro-OH	-----	-----
6	NH_2	-----	-----
7	OH	-----	-----
8	Pro	Lys	Gly-NH_2
9	Pro	Orn	Gly-NH_2
10	Pro	Dab	Gly-NH_2
11	Pro	Dap	Gly-NH_2
12	Pro	Cit	Gly-NH_2

Fig. 12

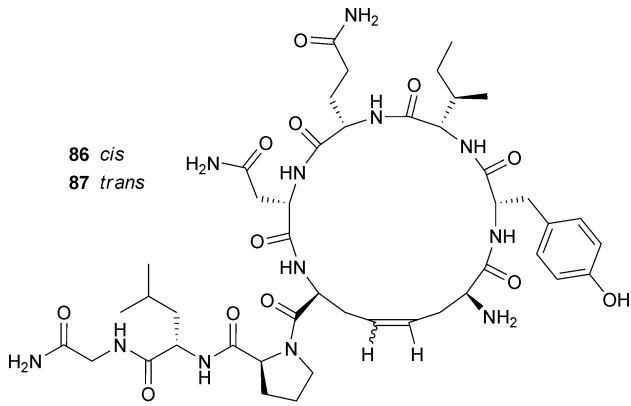
Seven new analogues of arginine vasopressin (AVP) substituted in position 2 or 3 with 1-aminocyclohexanecarboxylic acid (Acc) were prepared and their interaction with the vasopressin (VP) and oxytocin (OT) receptors ($V_{1A}R$, V_2R and OTR) was investigated.[396] All peptides were synthesized and tested in bioassays for pressor and antidiuretic activities. Also their uterotonic activity *in vitro* has been measured. This work was aimed at checking the applicability of computational methods for predicting receptor–ligand interactions and makes a good basis for selecting the receptor amino acid residues involved in ligand binding. The results of this study suggest preferred geometries of new, potentially active AVP analogues.

Twelve new analogues whose structure (Fig. 12) derived from modifications of cyclo[(S)Pmp^1,D-Trp^2,Pen^6,Arg^8]oxytocin; (S)Pmp = β,β-(3-thiapentamethylene-β-mercaptopropionic acid)] were synthesized.[397] Truncated analogues of the upper peptide from the C-terminus were systematically prepared ending in either the free acid or the amide. The 1–8 amide was roughly as potent as the original peptide in the rat uterotonic assay *in vitro*, and the shorter amides were only somewhat weaker antagonists. All four acid analogues were weaker antagonists than the original, but still maintained rather high antagonistic potency. These findings suggest that, if these truncated acids form as metabolites *in vivo*, they may contribute to the overall biological effect of oxytocin antagonists and their contribution should be taken into account. In addition, five analogues were made by substituting Arg^8 of cyclo[(S)Pmp^1,D-Trp^2,Pen^6,Arg^8]oxytocin with Lys^8, Orn^8, Dab^8, Dap^8 and Cit^8. All of these analogues maintained high potency as OTAs in the uterotonic assay, although their activity was only about 1.5–3 times lower than the original. The most potent analogue in the uterotonic assay, the Dap^8 analogue, pA_2 = 8.53, had

weak pressor activity (pA_2 = 6.90) and no antidiuretic effect. Hence, the 1–6 acid is a potent OTA with pA_2 = 8.27 and no measurable effect in the pressor or antidiuretic tests and thus it is a pure oxytocin antagonist. This fact makes it an attractive candidate for further studies on inhibition of OT biological effects and on preterm labour.

Facile synthesis of *cis* (**86**) and *trans* (**87**) olefin analogues of oxytocin [*H*-cyclo(Cys–Tyr–Ile–Gln–Asn–Cys)–Pro–Leu–Gly–NH_2; disulfide bond is replaced by a CH:CH double bond] is achieved *via* ring-closing metathesis on a resin-bound linear precursor peptide, Fmoc–Agly–Tyr(*t*–Bu)–Ile–Gln(CPh_3)–Asn(CPh_3)–Agly–Pro–Leu–Gly–NH–Resin (Agly = 2-allylglycine).[398] Hydrogenation of the *cis* olefin derivative proceeds selectively to generate the previously reported saturated derivative (**88**). Biological testing on rat uterus strips shows that *cis* olefin derivative has an EC_{50} = 38 ng/mL (oxytocin has EC_{50} = 2.7 ng/mL), whereas saturated derivative and *trans* olefin derivative are less active.

86 *cis*
87 *trans*



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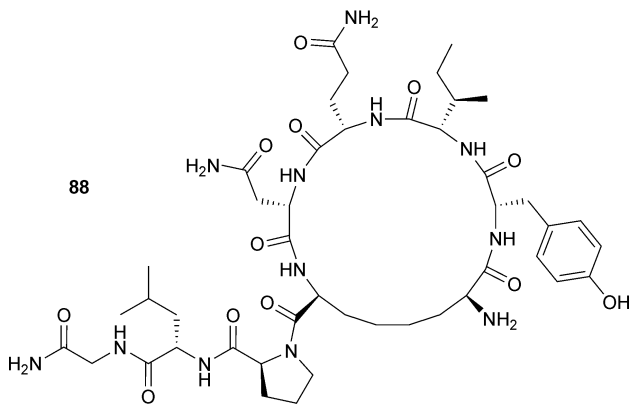


The synthesis and some pharmacological properties of seven new analogues of arginine vasopressin (AVP) substituted in position 2 or 3 with 1-aminocyclohexane-1-carboxylic acid (Acc) were described (Fig. 13).[399] All peptides were tested for the pressor, antidiuretic and uterotonic *in vitro* activities. The Acc^3 modifications of AVP, dAVP, [D-Arg^8]VP and [Cpa^1]AVP have been found to be deleterious for interaction with all three neurohypophyseal hormone receptors, as judged from the several orders of magnitude decreased biological activities, whereas Acc^2 substitution selectively altered the interaction with the receptors. Two of the new analogues, [Acc^2]AVP and [Acc^2, D-Arg^8]AVP, are potent antidiuretic agonists. [Acc^2]AVP exhibits moderate pressor agonistic activity and weak antiuterotonic properties. [Acc^2, D-Arg^8]AVP has been found to be a weak antagonist in the pressor and

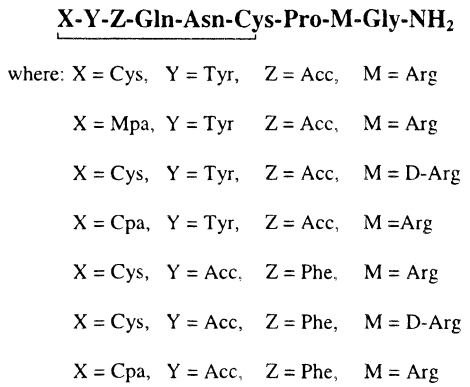


Fig. 13

uterotonic tests. Another analogue-[Cpa[1], Acc[3]]AVP—turned out to be a highly selective V_2 agonist. This is an unexpected effect, as its parent peptide, [Cpa[1]]AVP is a very potent V_{1A} receptor antagonist. This is the first Cpa[1] modification to have resulted in V_2 agonism enhancement. Besides providing useful information about structure–activity relationships, these results could open up new possibilities in the design of highly potent and selective V_2 agonists.

A series of 4′-[(4,4-difluoro-5-methylidene-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)carbonyl]benzanilides [R_1 = Cl, O_2N, Me, MeO, EtO, Me_2CHO, Ph; R_2 = H, MeO; R_3 = H_2N, MeNH, Et_2N, morpholino, 4-(dimethylamino)piperidino, *etc*.] was synthesized and evaluated for arginine vasopressin (AVP) antagonistic activity.[400] Compounds with alkoxy groups (especially ethoxy group) at the 2′-position of benzanilide possessed potent affinity and selectivity for the V_{1A} receptor *vs*. V_2 receptor. Further study has shown that the introduction of 4-(dimethylamino)piperidino and 4-morpholino groups at carbonylmethylene exhibited more potent affinity and selectivity for V_{1A} receptors.

Novel tricyclic benzazepine derivatives {R = phenanthridine-5-yl, dibenz[b,f]-[1,4]oxazepine-10-yl, pyrrolo[2,1-*c*][1,4]benzodiazepine-10-yl, *etc*., X = 2-Me} were synthesized as arginine vasopressin (AVP) antagonists.[401] Several tricyclic compounds showed potent antagonistic activity in rat AVP receptors V_{1A} and V_2. Derivatives containing pyrrolo-tricyclic amines, {R = 4,5-dihydropyrrolo[1,2-*a*]quinoxaline-5-yl, 10,11-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-10-yl, X = 2-Me; 6,7-dihydro-5*H*-pyrrolo[1,2-*a*][1,5]benzodiazepine-7-yl, X = 2-Me, 2-Me-3-F, 2-Me-5-F}, also showed selectivity for the V_2 receptor.

A series of novel 3,4,5,6-tetrahydro-1*H*-azepino[4,3,2-*cd*]indoles was synthesized and tested for vasopressin receptor antagonist activity.[402] The best compound bound to V_2 receptors with an IC_{50} value of 20 nM, had >100-fold selectivity over V_{1A} receptors, and inhibited cAMP formation in a cellular V_2 functional assay with an IC_{50} value of 70 nM.

4.16 Insulins and chemokines

4.16.1 Insulins. Insulin receptor substrate (IRS)-2 has been implicated in the promotion of β-cell survival. The novel analogue [LysB3, GluB29] insulin (insulin glulisine, IG)[403] might mediate an enhanced β-cell protective effect due to its unique property of preferential IRS-2 phosphorylation. The authors assessed IRS activation by IG and its anti-apoptotic activity against cytokines or palmitic acid in comparison to insulin, insulin analogues, and insulin-like growth factor (IGF)-I using INS-1 cells. IG induced a prominent IRS-2 activation without significant IRS-1 stimulation. In conclusion, the prominent anti-apoptotic activity of insulin glulisine might serve to counteract autoimmune- and lipotoxicity-induced β-cell destruction.

Insulin glargine is the first long-acting basal insulin analogue indicated for s.c. administration once daily at bedtime in adults with type 1 or type 2 diabetes mellitus and pediatric patients aged $\geq$ 6 year with type 1 diabetes. It differs in structure from native human insulin by 3 amino acids. In a review the systematic clinical investigation of this analogue was discussed.[404]

4.16.2 Chemokines. Peptide T, named for its high threonine content (ASTTTNYT), was derived by a database search which assumed that a relevant receptor binding epitope within env (gp120) would have sequence homology to a known signaling peptide. Binding of radiolabeled gp120 to brain membranes was displaced by peptide T and three octapeptide analogues (including "DAPTA", DAla^1-peptide T-amide, the protease-resistant analogue now in Phase II clinical trials) with the same potency that these four octapeptides blocked infectivity of an early passage patient isolate. Peptide T and analogues of its core pentapeptide, present near the V2 stem of numerous gp120 isolates, are potent ligands for CCR5.[405] The chemokine receptor CXCR4 is a co-receptor for T-tropic strains of HIV-1. A number of small molecular antagonists of CXCR4 are in development but all are likely to lead to adverse effects due to the physiological function of CXCR4. To prevent these complications, allosteric agonists may be therapeutically useful as adjuvant therapy in combination with small molecular antagonists. Two peptides, designated RSVM and ASLW, were identified as novel agonists that are insensitive to the CXCR4 antagonist AMD3100.[406] A hexapeptide, Ac–RRWWCR–NH_2 (antileukinate), has been reported to be a potent inhibitor of CXC–chemokine receptor. This study was undertaken to investigate the effect of s.c. administered antileukinate on experimentally bleomycin-induced acute lung injury in mice, in which CXC–chemokines have been reported to be involved. These findings suggest that antileukinate is able to inhibit acute lung injury by suppressing neutrophil mobilization induced by CXC–chemokines.[407] CXC chemokines bearing the glutamic acid–leucine–arginine (ELR) motif are crucial mediators in neutrophil-dependent acute inflammation. In this study, the authors measured the concentrations of these chemokines in human milk, sought their presence in human mammary tissue by immunohistochemistry, and confirmed chemokine expression in cultured human mammary epithelial cells.[408] An invention discloses peptides isolated from the extracellular domain of OX40 ligand (OX40L) capable of binding OX40 receptor (OX40R) and inhibiting OX40R-OX40L interaction. Such peptides, fusion proteins comprising them, as well as peptides and other molecules designed on their sequences, can be used as OX40R binding agents competing with natural OX40L for blocking OX40R-mediated cell signaling in the prophylaxis and/or treatment of diseases related to activated T cells. In particular, peptides are derived from the extracellular domain (P5, corresponding to residue 94–124, P5-1a:107–111, P5-1:107–116) of OX40L (also known as CD134 antigen ligand), that interact with OX40R with high affinity and compete with OX40L. This binding activity has been tested using *in vitro* assays employing recombinant forms of OX40R (OX40R-IgG1) and OX40L (OX40L-CD8). It is demonstrated that OX40L can be effectively competed by the claimed peptide sequences.[409] Libraries of linear and cyclic peptides (containing natural and unnatural amino acids) demonstrate enhanced binding affinity for CXCR4 and duration of action *in vivo* resulting from resistance to proteolysis.[410]

4.17 Peptide toxins

Seven cDNAs encoding six toxins HWTX-I, HWTX-II, HWTX-IIIa, HWTX-IV, HWTX-V, HWTX-VII and one lectin SHL-I, from the spider *Selenocosmia huwena*, were cloned and sequenced.[411] All of the cDNAs of these seven peptides encode a precursor including a potential signal peptide of 21–24 residues, a mature toxin of about 30 residues and an intervening pro region. The prepro regions of HWTX-I, HWTX-IIIa, HWTX-IV, HWTX-V and SHL-I were demonstrated, by the

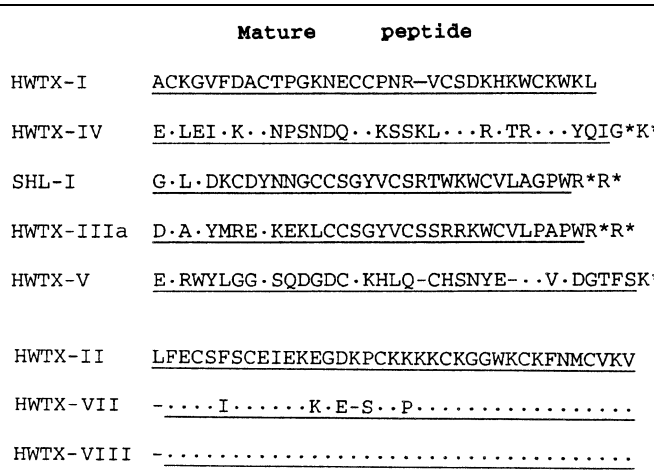


Fig. 14

comparison of the cDNA sequences, to have high similarity, which is concert with the similar inhibitor cystine knot motif of HWTX-I, HWTX-IV and SHL-I although their functions are different. It was also demonstrated that, HWTX-II and HWTX-VII share the highly similar prepro region which is different from that of HWTX-I, HWTX-IV and SHL-I. The three dimensional structure of HWTX-II has been detected to exhibit a different motif. This indicates that the seven peptides from *S. huwena* could be classified into two different superfamilies according to the prepro region of cDNA sequences. The primary structures of the upper toxins are shown in Fig. 14.

Venomous animals have evolved a vast array of peptide toxins for prey capture and defense. These peptides are directed against a wide variety of pharmacological targets making them an invaluable source of ligands for studying the properties of these targets in different experimental paradigms.[412] A number of these peptides have been used *in vivo* for proof-of-concept studies, with several having undergone preclinical or clinical development for the treatment of pain, diabetes, multiple sclerosis and cardiovascular diseases. The authors survey the pharmacology of venom peptides and assess their therapeutic prospects. Three peptide toxins (gigantoxins I–III) (Fig. 15) with crab toxicity were isolated from the sea anemone *Stichodactyla gigantea* by gel filtration and reverse-phase HPLC and their complete amino acid sequences were determined.[413] Gigantoxins II (44 residues) and III (48 residues) have LD_{50} (against crabs) of 70 and 120 microgram/kg, respectively, and are analogous to the known type 1 and 2 sea anemone sodium channel toxins, respectively. On the other hand, gigantoxin I (48 residues) is potently paralytic to crabs (ED_{50} 215 microgram/kg), although its lethality is very weak ($LD_{50} > 1000$ microgram/kg). Interestingly, gigantoxin I has 31–33% homologies with mammalian epidermal growth factors (EGFs), with the same location of six cysteine residues. In accordance with the sequence similarity, gigantoxin I exhibits EGF activity as evidenced by rounding of A431 cells and tyrosine phosphorylation of the EGF

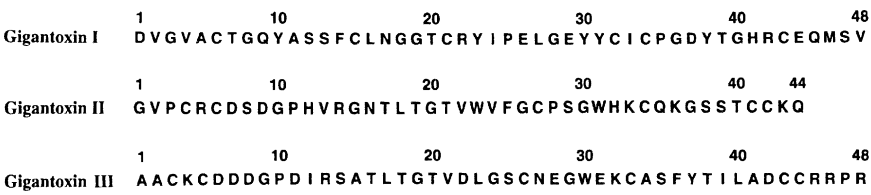


Fig. 15

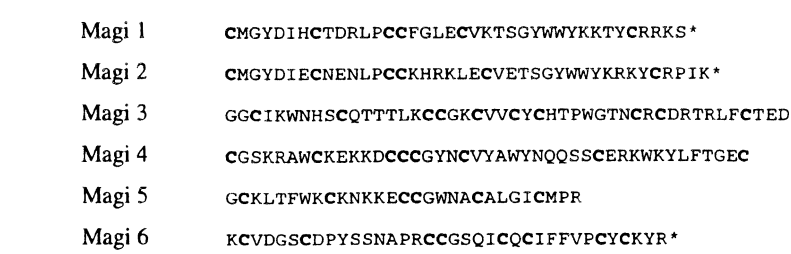


Fig. 16

receptor in the cells, although much less potently than human EGF. Gigantoxin I is the first example of EGF-like toxins of natural origin.

From two species of sea anemones, *Dofleinia armata* and *Entacmaea ramsayi*, three peptide toxins (two from the former and one from the latter) with crab toxicity were purified and completely sequenced.[414] The three toxins (30–32 residues) are highly homologous to each other and also to PaTX from *Entacmaea actinostoloides*, a type 3 sea anemone sodium channel toxin. This study reveals that there is a family of PaTX-like toxins in sea anemones.

Six peptide toxins (Magi 1–6) were isolated from the Hexathelidae spider *Macrothele gigas*.[415] The amino acid sequences of Magi 1, 2, 5 and 6 have low similarities to the amino acid sequences of known spider toxins. The primary structure of Magi 3 is similar to the structure of the palmitoylated peptide named PlTx-II from the North American spider *Plectreurys tristis* (Plectreuridae). Moreover, the amino acid sequence of Magi 4, which was revealed by cloning of its cDNA, displays similarities to the Na^+ channel modifier delta-atracotoxin from the Australian spider *Atrax robustus* (Hexathelidae). Competitive binding assays clearly demonstrated the specific binding affinity of Magi 1–5 to site 3 of the insect sodium channel and also that of Magi 5 to site 4 of the rat sodium channel. Magi 5 is the first spider toxin with binding affinity to site 4 of a mammalian sodium channel. The amino acid sequences *M. gigas* peptides are shown in Fig. 16.

A novel toxin, named C119, was isolated from the venom of the scorpion *Centruroides limpidus limpidus Karsch*. It is composed of 63 amino acid residues closely packed by four disulfide bridges.[416] It showed no apparent effect when injected to insects, crustaceans and *i.p.* to mice. However, when *i.c.v.* injected in the rat it immediately induced sleep, suggesting that it has a neurodepressant effect. This peptide is synthesized as a precursor of 84 amino acid residues and processed by removing 19 amino acids (signal peptide) from the amino terminal region and a couple of lysine residues from the carboxyl end. The presence of an intron of 777 bases interrupting the region encoding the signal peptide was also revealed. A comparison of its primary sequence, with more than 100 scorpion toxins known, showed that together with toxin CsE9 they constitute a new subfamily of peptides considered to be one of the most divergent groups of scorpion toxin-like peptides discovered. The sequence of C119 is the following: EDGYLFDKRK RCTLACIDKT GDKNCDTNCK KEGGSFGHCS YSACWCKGLP GSTPISRTPG KTC.

Three 26 kDa proteins, named as TJ-CRVP, NA-CRVP1 and NA-CRVP2, were isolated from the venoms of *Trimeresurus jerdonii* and *Naja atra*, respectively.[417] The N-terminal sequences of TJ-CRVP and NA-CRVPs were determined. The sequence aligment of CRVPs from snake venom is shown in Fig. 17. These components were devoid of the enzymatic activities tested, such as phospholipase A_2, arginine esterase, proteolysis, L-amino acid oxidase, 5′-nucleotidase, acetylcholinesterase. Furthermore, these three components did not have the following biological activities: coagulant and anticoagulant activities, lethal activity, myotoxicity, hemorrhagic

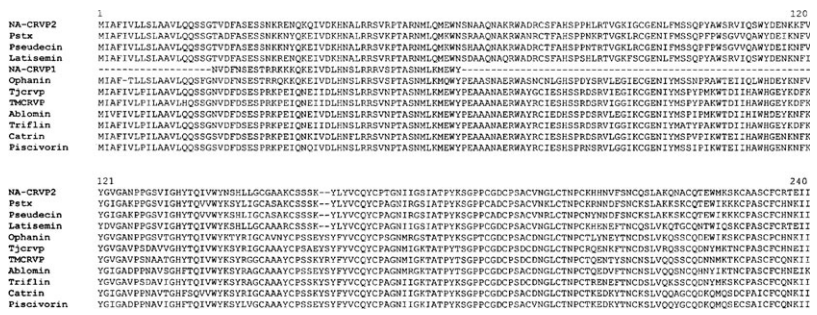


Fig. 17

activity, platelet aggregation and platelet aggregation-inhibiting activities. These proteins are named as cysteine-rich venom protein (CRVP) because their sequences showed high level of similarity with mammalian cysteine-rich secretory protein (CRISP) family. Recently, some CRISP-like proteins were also isolated from several different snake venoms, including *Agkistrodon blomhoffi, Trimeresurus flavoviridis, Lanticauda semifascita* and king cobra.

Hainantoxin-I is a novel peptide toxin, purified from the venom of the Chinese bird spider *Selenocosmia hainana* (= *Ornithoctonus hainana*). It includes 33 amino acid residues with a disulfide linkage of I–IV, II–V and III–VI, assigned by partial reduction and sequence analysis.[418] Under two-electrode voltage-clamp conditions, hainantoxin-I can block $rNa_v1.2/\beta_1$ and the insect sodium channel para/tipE expressed in *Xenopus laevis* oocytes with IC50 values of $68+/-6$ μM and $4.3+/-0.3$ μM, respectively. The structure comparison of hainantoxin-I to other related toxins are shown in Fig. 18. The three-dimensional solution structure of hainantoxin-1 belongs to the inhibitor cystine knot structural family determined by two-dimensional ^{1}H-NMR techniques. Structural comparison of hainantoxin-I with those of other toxins suggests that the combination of the charged residues and a vicinal hydrophobic patch should be responsible for ligand binding. This is the first report of an insect sodium channel blocker from spider venom and it provides useful information for the structure–function relationship studies of insect sodium channels.

K^+ channels are macromolecules embedded in biological membranes, where they play a key role in cellular excitability and signal transduction pathways. Knowledge of their structure should help improve our understanding of their function and lead to the design of therapeutic compounds. Most pharmacological and structural characteristics of these channels have been elucidated by using high-affinity channel blockers isolated from scorpion venoms.[419] The activities of scorpion toxins specific for K^+ channels are summarized in Table 1. Recent data on the three-dimensional structures of K^+ channels and novel scorpion toxins suggest a variety of novel interacting modes of these channels and toxins, which should help increase our understanding of the K^+ channel structure–function relationship.

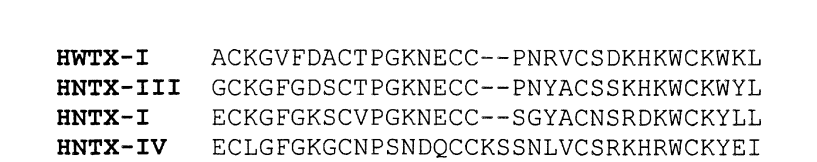


Fig. 18

Table 1

Toxin	Channels tested	K_d (nm)	Toxin	Channels tested	K_d (nm)
Charybdotoxin/α-KTx 1.1	*Shaker* (d)	120	Maurotoxin/α-KTx 6.2	*Shaker* (d)	2.4
	K_v1.3 (r)	0.17		K_v1.2 (m)	0.12
	K_{Ca}1.1(r)	2.1		K_v1.3 (h)	150
	K_{Ca}3.1 (h)	5		K_{Ca}2.2 (h)	High[e]
Iberiotoxin/α-KTx 1.3	K_{Ca}1.1 (b)	1.7		K_{Ca}2.3 (h)	High
Noxiustoxin/α-KTx2.1	*Shaker* (d)	160		K_{ca}3.1 (h)	1.1
	K_v1.1 (m)	24	Pi2/α-KTx 7.1	K_v1.1 (d)	8.3
	K_v1.3 (r)	0.31		K_v1.3 (h)	0.044
	K_{Ca}1.1 (r)	450	PO1/α-KTx 8.1	K_{Ca}2.2 (h)	High
Margatoxin/αKTx 2.2	*Shaker* (d)	160		K_{Ca}2.3 (h)	High
	K_v1.3 (h)	0.03	BmPO2/α-KTx 9.1	I_{TO} (r)	NA[f]
Kaliotoxin/α-KTx 3.1	K_v1.3 (r)	0.41	Cobatoxin/α-KTx 10.1	*Shaker* (d)	700
Agitoxin 2/α-KTx 3.2	*Shaker* (d)	0.16		K_v1.1 (h)	500
	K_v1.1 (r)	0.044	PbTx1/α-KTx 11.1	NA[f]	—
	K_v1.3 (r)	0.004	Butantoxin/α-KTx 12.1	*Shaker* (d)	660
TsKα/αKTx 4.1	K_v1.2 (m)	0.2	Tc1/α-KTx 13.1	*Shaker* (d)	65
TsK/α-KTx 4.2	K_{Ca}2.2 (h)	80	OsK2/α-KTx 13.2	K_v1.1 (r)	High
	K_{Ca}2.3 (h)	197		K_v1.2 (r)	97
Scyllatoxin/α-KTx 5.1	K_{Ca}2.2 (h)	0.3	BmKK1/α-KTx 14.1	NA[f]	—
	K_{Ca}2.3 (h)	1.1	Aa1/α-KTx 15.1	*Shaker* (d)	High
PO5/α-KTx 5.2	K_{Ca}2.2 (h)	22		1_A (r)	150
	K_{Ca}2.3 (h)	25	Martentoxin/α-KTx 16.2	K_{Ca}1.1 (r)	21
Tamapin/α-KTx 5.4	K_{Ca}2.1 (h)	42	TXKs4/α-KTx 17.1	NA[f]	—
	K_{Ca}2.2 (r)	0.02	Tc32/α-KTx 18.1	*Shaker* (d)	High
	K_{Ca}2.3 (r)	1.7		K_v1.3 (h)	10
Pi1/α-KTx 6.1	*Shaker* (d)	32	Ergtoxin/γ-KTx 1.1	I_{ERGs} (m)	16
	K_v1.3 (h)	11		K_v11.1 (h)	6.5
	K_{Ca}2.2 (h)	100	BemK-1/γ-KTx 2.1	K_v11.1 (h)	6.2
	K_{Ca}2.3 (h)	250	CnErg2/γ-KTx 3.1	1_{ERGs} (r)	418
			CsEKerg1/γ-KTx 4.12	1_{ERGS} (m)	230
			CsErg5/γ-KTx 5.1	NA[f]	—

b, cattle; d, fruit fly; h, human; m, mouse; r, rat.

Voltage-dependent potassium channel Kv2.1 is widely expressed in mammalian neurons and was suggested to be responsible for mediating the delayed rectifier (IK) currents. Further investigation of the central role of this channel requires the development of specific pharmacophores, for instance, the utilization of spider venom toxins.[420] Most of these toxins belong to the same structural family with a short peptide reticulated by disulfide bridges and share a similar mode of action. The primary structures of these related tarantula toxins are shown in Fig. 19. Hanatoxin 1 (HaTx1) from a Chilean tarantula was one of the earliest discussed tools regarding this and has been intensively applied to characterize the channel blocking not through the pore domain. Recently, more related novel toxins from African tarantulas such as heteroscordratoxins (HmTx) and stromatoxin 1 (ScTx1) were isolated and shown to act as gating modifiers such as HaTx on Kv2.1 channels with electrophysiological recordings. However, further interaction details are unavailable due to the lack of high-resolution structures of voltage-sensing domains in such mammalian Kv channels. Therefore, in the present study, the authors explored structural observation *via* molecular docking simulation between toxins and Kv2.1 channels based upon the solution structures of HaTx1 and a theoretical basis of an individual S3C helical channel fragment in combination with homology modeling

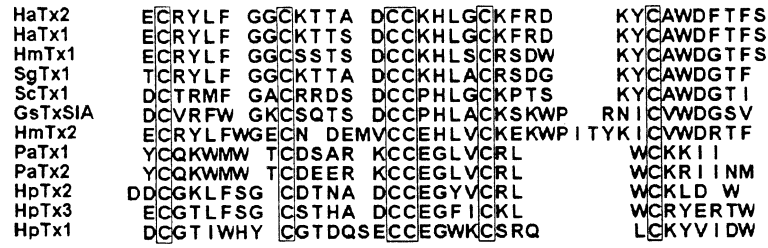


Fig. 19

for other novel toxins. The results provide precise chemical details for the interactions between these tarantula toxins and channel, reasonably correlating the previously reported pharmacological properties to the three-dimensional structural interpretation.

Advances in mass spectrometry and peptide biochemistry coupled to modern methods in electrophysiology have permitted the isolation and identification of numerous novel peptide toxins from animal venoms in recent years.[421] These advances have also opened up the field of spider venom research, previously unexplored due to methodological limitations. Many peptide toxins from spider venoms share structural features, amino acid composition and consensus sequences that allow them to interact with related classes of cellular receptors. They have become increasingly useful agents for the study of voltage-sensitive and ligand-gated ion channels and the discrimination of their cellular subtypes. (Spider toxins, their sources and targets are summarized in Table 2.) Spider peptide toxins have also been recognized as useful agents for their antimicrobial properties and the study of pore formation in cell membranes. Spider peptide toxins with nanomolar affinities for their receptors are thus promising pharmacological tools for understanding the physiological role of ion channels and as leads for the development of novel therapeutic agents and strategies for ion channel-related diseases. Their high insecticidal potency can also make them useful probes for the discovery of novel insecticide targets in the insect nervous system or for the development of genetically engineered microbial pesticides. Table 3 shows the sequences of spider toxins affecting ion channels, glutamate uptake and phospholipid membranes.

5. Enzyme inhibitors

A number of reviews were published in 2003 on enzyme inhibitors. The therapeutic potential and the neuroprotective effect of calpain inhibitors are summarized[422,423] as well as the possibility of their use for prevention of neurodegeneration. Lysosomal cysteine proteases (cathepsins) and their inhibitor as putative drug discovery target are reviewed.[424,425] Another publication gives insight into the roles of cathepsins in antigen processing and presentation revealed by specific inhibitors.[426] Angiotensin-1-converting enzyme (ACE) and its relatives[427] as well as ACE-inhibitors are reviewed.[428] Another scientific paper dealt with the potential of renin inhibition in cardiovascular disease.[429] The mevalonate synthesis pathway, farnesyltransferase as a therapeutic target in cancer is reviewed.[430] Strategies in the design of prodrugs as anti-HIV agents are summarized.[431] The role of NO in angiogenesis[432] and the regulation of inducible nitric oxide synthase by cAMP-elevating phosphodiesterase inhibitors[433] are reviewed. A special review deals with the interruption of tumor cell cycle progression through proteasome inhibition.[434] The molecular design and biological activities of protein-tyrosine phosphatase inhibitors are summarized.[435] Human β-secretase (BACE) inhibitors represent an important field of drug design for treatment of Alzheimer's disease.[436] Specialized reviews deal with the use of direct thrombin inhibitors for anticoagulation.[437,438] Very intensive work with

Table 2

Spider	Peptide	Target	Spider	Peptide	Target
Suborder Mygalomorphae family Theraphosidae			**Suborder Araneomorphae family Agelenidae**		
Grammostola spatula	HaTx1,2	K^+	*Agelenopsis aperta*	ω-Agal – IVA	Ca^{2+}
	VSTx1			μ-agatoxin 1-6	Na^+
	GsMTx2,4	MS	*Hololena curta*	Curtatoxin I-III	Na^+
	GSTxSIA	Ca^{2+}	*Puracoelotes luctuosus*	δ-PaluITI -4	Na^+
		K^+	*Tegenaria agrestis*	TaITx1-3	?
Phrixotrichus auratus	PaTx1, 2	K^+	**Family Segestriidae**		
Stromatopelma calceata	ScTx1	K^+	*Segestria florentina*	SNX-325	Ca^{2+}
Heteroscodra maculata	HmTx1, 2	K^+	**Family Diguetidae**		
Scodra griseipes (now Stromatopelma calceata)	SGTx1	K^+	*Diguetia canities*	DTX9.2	?
Thrixopelma pruriens	ProTxI, II	Na^+	**Family Filistatidae**		
			Filistada hibernalis	DW13.3	Ca^{2+}
Psalmopoeus cambridgei	PcTx1	ASIC	**Family Ctenidae**		
Selenocosmia huwena (now Ornithoctonus huwena)	Huwentoxin I huwentoxin IV	Ca^{2+} Na^+	*Phoneutria nigriventer*	PhTx4(6-1)	Na^+
Hysterocrates gigas	SNX-482	Ca^{2+}		PnTX3-4	GU
Acanthoscurria gomesiana	Gomesin	PLM		PhTx3-1	K^+
				ω-PTxIIA	Ca^{2+}
Family Hexathelidae			*Cupiennus salei*	Cupiennin 1-4	PLM
Hadronyche versuta	ω-ACTX-Hvla	Ca^{2+}	**Family Plectreuridae**		
	ω-ACTX-Hv2a	Ca^{2+}	*Plectreurys tristis*	PITxI-VIII	Ca^{2+}
	δ-ACTX-Hv1	Na^+	**Family Lycosidae**		
	J-HCTX-Hv1c	?	*Lycosa carolinensis*	lycotoxinI, II	PLM
Atrax robustus	δ-ACTX-ArI	Na^+	**Family Oxyopidae**		
			Oxyopes kitabensis	Oxyopinin 1, 2a, b	PLM
Family Cyrtaucheniidae			**Family Sparassidae**		
Aptostichus schlingeri	ApTxs	?	*Heteropoda venatoria*	HpTx1-3	K^+

Table 3

Name	Amino acid sequences	Toxin activity
Voltage-gated K^+ *blockers* Hanatoxin 1	ECRYLFGGCKTTSDCCKHLGCKFRDKYCAWDFTFS	Kv2.1 (shab-related) and shab1-related channels expressed in *Xenophus laevis* oocytes
Heteropodatoxin 1	DCGTIWHYCGTDQSECCEGWKCSRQLCKYVIDW	Kv4.2 expressed in *X. laevis* oocytes
Phrixotoxin 1	YCQKWMWTCDSARKCCEGLVCRLWCKKII	Kv4.2 and Kv4.3 in COS transfected cells and in *X. laevis* oocytes
ScTx1	DCTRMFGACRRDSDCCPHLGCKPTSKYCAWDGTI	Kv2.1 and Kv2.2 in COS transfected cells
HmTx1	ECRYLFGGCSSTSDCCKHLSCRSDWKYCAWDGTFS	Kv4 and Kv4.1 in COS transfected cellS
SGTx1	TCRYLFGGCKTTADCCKHLACRSDGKYCAWDGTF	Fast transient and delayed reactifier currents in rat cerebellum granular cells
PhTx3-1	AECAAVYERCGKGYKRCCEERPCKCNIVMDNCTCKKFISE	A-type currents in GH3 cells
Ca^{2+} blockers	AKALPPGSVCDGNESDCKCYGKWHKCRCPWKWH	L-type in rat dorsal root gang-lion neurons
ω-AgaIA	FTGEGPCTCEKGMKHTCITKLHCPNKAEW GLDW-SPC	
ω-AgaIIA	GCIEIGGDCDGYQEKSYCQCCRNNGFCS. . .Incomplete	N-type in chick synaptosomes
ω-AgaIIIA	SCIDIGGDCDGEKDDCQCCRRNGYCSCYSLFGY LKSGCKCVVGTSAEFQGICRRKARQCYNSDP DKCESHNKPKRR	L-, P/Q-, R-, N-type in rat brain synaptosomes and HEK293 transfected cells
ω-AgaIVA	KKKCIAKDYGRCKWGGTPCCRGRGCICSIMGTN CECKPRLIMEGLGLA	P/Q-type and P-type currents in cerebellar Purkinje neurons
SNX-482	GVDKAGCRYMFGGCSVNDDCCPRLGCHSLFSYC AWDLTFSD	R-type current in rat neurohypophyseal nerve terminals and L-type calcium channel in transfected HEK cells
SNX-325	GSCIESGKSCTHSRSMKNGLCCPKSRCNCRQIQ HRHDYLGKRKYSCRCS	N-type expressed in *X. laevis* oocytes
GSTxSIA	DCVRFWGKCSQTSDCCPHLACKSKWPRNI CVWDGSV	N- and P/Q-type in rat hippo-campal neurons
Huwentoxin I	ACKGVFDACTPGKNECCPNRVCSDKHKWCKWKL	N-type expressed in prostaglandin E1-differentiated NG108-15 cells
DW13.3	AECLMIGDTSCVPRLGRRCCYGAWCYCDQQLSC RRVGRKRECGWVEVNCKCGWSWSQRIDDWRAD YSCKCPEDQ	P/Q-, N-, L-, and R-type in *X. laevis* oocytes
ω-ACTX-Hv1a	SPTCIPSGQPCPYNENCCSQSCTFKENE NGNTVKRCD	VSCC in abdominal ganglia neurons of cockroach *Periplaneta americana*

Table 3 *(continued)*

Name	Amino acid sequences	Toxin activity
ω-ACTX-Hv2a	LLACLFGNGRCSSNRDCCELTPVCKRGSCVSSG PGLVGGILGGIL	VSCC in neurons of honeybee *Apis mellifera*
ω-phonetoxin IIA	SCINVGDFCDGKKDDCQCCRDNAFCSCSVIFGY KTNCRCEVGTTATSYGICMAKHKCGRQTTC TKPCLSKRCKKNH	P/Q-, N-, and R-type in BHK cell lines
ω-PITxII	ADCSATGDTCDHTKKCCDDCYTCRCGTPWGANC RCDYYKARCDT-Pa	Blocks insect pre-synaptic Ca^{2+}
Na^+ blockers	ECVPENGHCRDWY – DECCEGFYCSCRQP PKCICRNNN	Induces repetitive firing of action potentials in ventrolateral muscles of *Musca domestica*
μ-Agatoxin I		
Curtatoxin I	SCVGEYGRCRSAY – EDCCDGYYCNCSQP PYCLCRNNN	Induces repetitive firing of action 76 potentials in ventrolateral muscles of *Musca domestica*
δ-Palutoxin I	GCLGEGEKCADWSGPSCCDGFYCSCRSM PYCRCRNNS	Blocks inactivation of Na^+ currents in cockroach *P. Americana* axon
Robustoxin	CAKKRNWCGKNEDCCCPMKCIYAWYNQQGSCQTTITGLFKKC	Blocks inactivation of Na^+ currents in adult rat dorsal root ganglion neurons
Versutoxin	CAKKRNWCGKTEDCCCPMKCVYAWYNEQGSCQS TISALWKKC	Blocks inactivation of Na^+ currents in adult rat dorsal root ganglion neurons
PhTx2-6	ATCAGQDQPCKETCDCCGERGECVCGGPCICRQ GYFWIAWYKLANCKK	Modifies Na^+ currents in frog *Rana catesbiana* skeletal muscle
PhTx4(6-1)	CGDINAACKEDCDCCGYTTACDCYWSKSCKCRE AAIVIYTAPKKKLTC	Blocks inactivation of Na^+ currents in cockroach *P. Americana* axon
Huwentoxin IV	ECLEIFKACNPSNDQCCKSSKLVCSRKTRWCKYQI	Blocks the pore of Na^+ channels in adult rat dorsal root ganglion neurons
ProTxI	ECRYWLGGCSAGQTCCKHLVCSRRHGWCVWDGTFS	Nav1.8 and Kv2.1 expressed in *X. laevis* oocytes, and T-type in HEK293 cells
ProTxII	YCQKWMWTCDSERKCCEGMVCRLWCKKKW	Nav1.8 and Kv2.1 expressed in *X. laevis* oocytes
Ligand-gated	EDCIPKWKGCVNRHGDCCEGLECWKRRRSFEVC VPKTPKT	ASIC in sensory neurons from rat and ASIC 1a expressed in *X. laevis* oocytes and COS cells

Table 3 *(continued)*

Name	Amino acid sequences	Toxin activity
PcTxI		
Mechano sensitive ion channels	GCLEFWWKCNPNDDKCCRPKLKCSKLFKLCNFSSA	Stretch-activated channels in adult rat astrocytes
GsMTx4		
Glutamate uptake	SCINVGDFCDGKKDCCQCDRDNAFCSCSVIFGY KTNCRCE	Inhibits glutamate uptake in rat synaptosomes
PnTX3-4		
Unknown mode of action	AICTGADRPCAACCPCCPGTSCKAESN GVSYCRKDEP	Unknown
J-ACTX-Hv 1c		
Pore-forming	IWLTALKFLGKHAAKHLAKQQLSKL	Phospholipids, promotes efflux of Ca^{2+} from synaptosomes, red blood cells
Lycotoxin I		
Lycotoxin II	KIKWFKTMKSIAKFIAKEQ MKKHLGGE	Phospholipids, promotes efflux of Ca^{2+} from synaposomes, red blood cells
Cupiennin I	GFGALFKFLAKKVAKTVAKQAAKQGAKYVVNKQME	Antimicrobial analysis, red blood cells
Oxyopinin I	FRGLAKLLKIGLKSFARVLKKVLPKAAKAGKAL AKSMADENAIRQQNQ	Phospholipids, reduction of cell membrane resistance by opening non-selective ion channels in Sf9 cells
Oxyopinin 2a	GKFSVFGKILRSIAKVFKGVGKVRKQF KTASDLDKNQ	Phospholipids, reduction of cell membrane resistance by opening non-selective ion channels, red blood cells
Gomesin	QCRRLCYKQRCVTYCRGR	Antimicrobial

topoisomerase inhibitors (rebeccamycin analogues)[439] and the use of telomerase inhibitors in cancer therapy[440,441] has been reviewed. Chymase inhibitors are shown as the next putative drug candidates for the treatment of cardiovascular disorders.[442] Aminoacyl-tRNA synthetases and their inhibitors as a novel family of antibiotics are reviewed.[443]

5.1 Aminopeptidase and deformylase inhibitors

The cell surface aminopeptidase N (APN/CD13), overexpressed in tumor cells, plays a critical role in angiogenesis. With the aim of developing a new generation of noncytotoxic APN/CD13 inhibitors a series of novel flavone-8-acetic acid derivatives was synthesized.[444] A new class of inhibitors of cytosol leucine aminopeptidase (LAP) was designed and prepared, the organophosphorous compounds were very potent.[445] 1-Aminoalkanephophonic acids were synthesized stereoselectively, the compounds acted as moderate inhibitors of LAP.[446] The exopeptidase specificity of aminopeptidase-A was studied with molecular modeling and site-directed mutagenesis.[447] Aminopeptidase-C[448] and puromycin-sensitive aminopeptidase[449] were measured using fluorogenic substrates for continuous fluorometric assay and visualization, respectively. Potent, specific, chemically stable and non-peptide/small-molecular inhibitors of puromycin-sensitive aminopeptidase with a 2,4(1*H*,3*H*)-quinazolinedione skeleton were synthesized.[450]

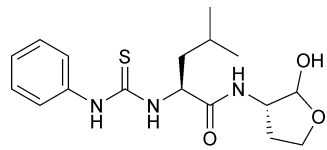
Mutation of 3 amino acids in the active site of the puromycin-sensitive aminopeptidase converted the enzyme into a catalytically inactive binding protein.[451] Methionine aminopeptidases (MetAPs) are a unique class of cobalt-containing metalloproteinases existing both in prokaryotic and eukaryotic cells. A series of pyridine-2-carboxylic acid derivatives were synthesized and potent MetAP inhibitors have been obtained.[452] Fumagillin, a known natural compound with antiangiogenic activity reversibly inhibits also the catalytic activity of MetAP.[453] A new technology, proteolysis targeting chimeric molecule (Protac) was used for ubiquitination and proteasomal degradation of MetAP-2 for inhibiting protein activity[454] and suppression of tumor. MetAP-2, a novel target for cancer therapy, and A-357300, a novel inhibitor, were prepared after rational drug design for reversible inhibition of MetAP-2 activity.[455]

The aminopeptidase from Aeromonas proteolytica (AAP) is a co-catalytic metallopeptidase and serves as a tool for studying the active site of other metallopeptidases that represent pharmaceutical targets.[456] Another enzyme, the zinc metallopeptidase from *Plasmodium falciparum* is a potential target for antimalarial drugs. The design and synthesis of a library of 45 quinoline-based inhibitors of the enzyme was reported.[457] The mechanism of substrate recognition of prolyl aminopeptidase was studied using novel inhibitors containing Pro, Ala or Sar and 2-*tert*-butyl[1,3,4]oxadiazole.[458]

Deformylases belong to a subfamily of metalloproteases that catalyze the removal of the N-terminal formyl group from newly synthesized proteins. The structure of the known deformylase-inhibitor BB-3497 was modified and the structure–activity relationship was studied.[459] The analogues of the deformylase inhibitor VRC 3375, α-substituted hydroxamic acids were studied for antibacterial activity.[460] A human protein deformylase (PDF) was characterized and studied. Results suggest that human PDF is likely to be an evolutional remnant without any functional role, therefore bacterial PDF represents an excellent target for antibacterial drug design.[461] A macrocyclic, peptidomimetic inhibitor of peptide deformylase was designed; it contains *N*-formylhydroxylamine side chain as metal chelating group. The inhibitor showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria.[462]

5.2 Calpain inhibitors

Calpains (calcium-dependent thiol proteases) are expressed in ubiquitous and tissue specific isoforms. The crystal structure of calpain is already known. A novel membrane-permeable specific calpain inhibitor was developed by fusing calpastatin peptide and 11 poly-arginine peptide (11R) for effective penetration of the inhibitor across the plasma membrane of living neurons.[463] Novel benzoylalanine-derived ketoamides were prepared and studied for calpain-I inhibition. Derivatives carrying vinylbenzyl amino residues in the P_2–P_3 region inhibited calpain in nanomolar concentrations and thus, represented a novel class of nonpeptidic calpain inhibitors.[464] Four novel compounds were designed and synthesized based on the structure of the known potent calpain inhibitor, peptidyl aldehyde SJA6017.[44] One of the compounds, a cyclic hemiacetal (**89**) exhibited potent inhibitory activites, high cornea permeability and excellent efficacy in rat lens cataract model.[465]



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Structure–activity relationship and drug profile of *N*-(4-fluorophenyl-sulfonyl)-L-valyl-L-leucinal (SJA6017) were studied.[466]

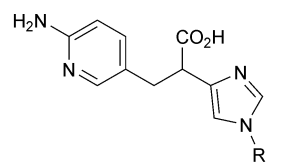
5.3 Carboxypeptidase inhibitors

Carboxypeptidase-A cleaves the C-terminal amino acid residue having an aromatic side chain. All four possible stereoisomers of 2-benzyl-3-methanesulfinylpropanoic acid were synthesized and evaluated as inhibitors for carboxypeptidase-A.[467] In the same laboratory, 2-ethyl-2-methyl-3-mercaptopropanoic acid and 2-benzyl-2-methyl-3-mercaptopropanoic acid were synthesized and evaluated as inhibitors for carboxypeptidase-A.[468] Thiol-based inhibitors of glutamate-carboxypeptidase II (GCPII) were synthesized and an orally active GCPII inhibitor was discovered.[469] A series of hydroxamic acid has been prepared as potential inhibitors of GCPII.[470] Structure–activity studies showed that the inhibitory potency was dependent on the number of methylene units between the hydroxamate group and pentanedioic acid.

The activated form of thrombin-activable fibrinolysis inhibitor (TAFIa, EC 3.4.17.20) proved to be a carboxypeptidase and was recently identified as an important regulator of fibrinolysis.[471] Appropriately substituted imidazole acetic acid (Fig. 20) were found to be potent inhibitors of the activated enzyme FAFIa and also some other carboxypeptidases (CPA, CPN, CPM).

5.4 Caspase inhibitors

The caspase family includes highly conserved cysteine proteases subdivided into 3 groups.



R = H, Me, Et, *i*Pr, Bu, Bn or *i*Pent

Fig. 20

Novel 3-alkoxy-7-amino-4-chloro-isocoumarin derivatives were synthesized and evaluated as inhibitors of several proteases, *e.g.* serine proteases and caspase-3. The new inhibitors are only weakly active on caspase-3, but prevent γ-secretase mediated production of Aβ 1–40/42 amyloid peptides.[472] Caspase cleavage of amyloid precursor protein (APP) has been suggested as leading process to increased production of Aβ peptides. However, more recently it was proven that caspase activation increases Aβ generation independently of caspase cleavage of APP.[473]

Caspase-3 lies at a key junction in the apoptotic cascade, mediating apoptosis from both the intrinsic and extrinsic activation pathways. Inhibition of caspase-3 may prove to be a valuable therapeutic approach for treating diseases involving Lys regulated apoptosis (*e.g.* stroke, traumatic brain injury, myocardial infarct, sepsis). A series of novel and potent small-molecule inhibitors of caspase-3 was designed and synthesized.[474] Another research group designed and synthesized a series of anilinoquinosolines, a new and structurally novel class of potent small molecule inhibitors of caspase-3.[475]

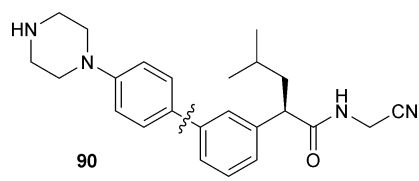
X-Chromosome-linked inhibitors of apoptosis protein (XIAP) are regarded as the most potent suppressors of mammalian apoptosis through direct binding and inhibition of caspases. A novel group of nonpeptidic small molecule inhibitors of the XIAP/caspase-3-interaction was developed and the activity was characterized both *in vitro* and in cells.[476]

The total synthesis of wedelolactone, a naturally occurring direct inhibitor of IKK complex was performed. The complex can suppress LPS-induced caspase-11 expression.[477]

5.5 Cathepsins and other cysteine-protease inhibitors

Cathepsins and other cysteine protease inhibitors have been grouped into families and clans. Calpain (together with cathepsins) is a member of the papain clan (clan CA). Caspases, legumain, *etc.* are members of clan CD. Calpain and caspase inhibitors were previously summarized in Chapters 5.2 and 5.4. Lysosomal cathepsins and their inhibitors as putative targets have been reviewed.[424,425]

A novel class of cathepsin B inhibitors has been developed with a 1,2,4-thiadiazole heterocycle as the thiol trapping pharmacophore.[478] Four new peptidyl aldehydes bearing proline mimetics at the P_2-position were synthesized and studied as inhibitors of cathepsin-B, calpain I and selected serine proteases.[479] Cathepsin K, a member of the papain superfamily has been implicated in the bone resorption process: it is highly expressed in osteoclasts (the cells responsible for bone resorption) and it is one of the few proteases that can efficiently hydrolyze native collagen. Selective inhibitors of cathepsin K could therefore be potential therapeutic agents for the treatment of excessive bone loss such as osteoporosis. Previously, a substituted biphenyl compound (**90**) has been identified as a potent, selective and reversible inhibitor of cathepsin K.



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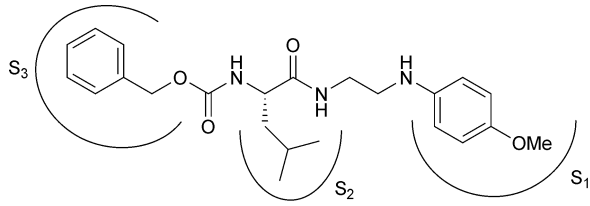
A large-scale, chromatography-free synthesis of the inhibitor is reported.[480] Another research group described molecular modeling of the lead compound (**91**) into the active site of cathepsin K and the synthesis and *in vitro* activities of a series of arylaminoethyl based inhibitors of the cathepsin K.[481]



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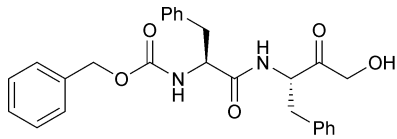
A novel series of 3,4-disubstituted azetidinone-based inhibitors of cathepsin K has been identified.[482] More recent data from kinetic and mass spectrometry experiments with these enzyme inhibitors are consistent with the interpretation that 3,4-disubstituted azetidin-2-ones transiently acylate the sulfhydryl of cathepsin K.[483] Novel nonpeptidic biaryl compounds were identified as potent and reversible inhibitors of cathepsin K.[484] The best compound of this series possesses an IC_{50} value of 3 nM for cathepsin K, that is selective *versus* cathepsins B (IC_{50} = 3950 nM), L (IC_{50} = 3725 nM) and S (IC_{50} = 2010 nM). The novel nonpeptidic inhibitor shows a fully reversible effect on cathepsin K. The results of the *in vivo* experiments with ovariectomized rhesus monkeys strongly suggest that inhibition of cathepsin K is a *via*ble therapeutic approach for the treatment of osteoporosis.[63] β-Lactam skeleton, a well known pharmacophore has been used for designing and synthesizing a new class of inhibitors for cathepsins B, L, K and S.[485] These inhibitors may interact with the nucleophilic thiol of the cysteine in the active site of cathepsins and are very potent inhibitors for cathepsins K, L and S at the nanomolar or subnanomolar IC_{50} values. A 65-amino acid residue polypeptide (MHC II-associated p^{41} invariant chain fragment, p^{41} icf) was synthesized using a combined solid-phase/solution approach.[486] CD, NMR and surface plasmon resonance were used for the structural and functional characterization of synthetic p^{41} icf, a potent inhibitor of human cathepsin L.

The growing number of resolved X-ray structures of cysteine-proteases as well as molecular modeling methods enables further development of potent inhibitors from lead structures (peptidic, peptidomimetic and non-peptidic leads). A review was focused on new non-peptidic cysteine protease inhibitors which have been developed during the last years.[487]

New drugs to combat malaria are urgently needed owing to the emergence of the widespread resistance of *Plasmodium falciparum* to available antimalarial drugs.[488] The cysteine proteases of the malaria parasite, falcipain-2 and falcipain-3 appear to be required for hemoglobin hydrolysis by intraerythrocytic parasites. A series of new peptidyl vinyl sulfone, vinyl sulfonate ester and vinyl sulfonamide derivatives has been designed and synthesized. Structure–activity studies showed that the best compounds inhibited *P. falciparum* development at low nanomolar concentrations. Falcipain inhibition correlates with antiparasitic activity and these results suggest that peptidyl vinyl sulfones have promise as antimalarial agents. Another antimalarial drug candidates, 1,4,7-trisubstituted isoquinolines were designed, synthesized and evaluated for their inhibition against *P. falciparum* cysteine proteinase falcipain-2.[489]

Other parasitic protozoan species of the genus Trypanosoma and Leishmania contain cysteine proteases and trypanothione reductase. These enzymes are attractive targets for the development of antitrypanosomal and antileishmanial agents. Recent development in the synthesis of low-molecular mass inhibitors of these enzymes is reviewed.[490] The protozoan parasite *Trypanosoma cruzi* infection results in Chagas' disease. Irreversible inhibition of the parasite's major cysteine protease, cruzain, can cure parasitic infections in mouse models. These results demonstrate the therapeutic promise of inhibitors of cruzain for the treatment of Chagas' disease. Now the crystal structures of two hydroxymethyl ketone inhibitors (**92** and **93**) complexed to the cysteine protease cruzain have been determined at 1.1 and 1.2 Å

resolution, respectively.[491] A series of compounds were prepared and tested based upon the structures providing further insight into the key binding interactions.



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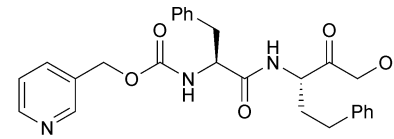

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5.6 Cytomegalovirus and rhinovirus 3C protease inhibitors

Human cytomegalovirus (HCMV) is a ubiquitous human pathogen responsible for several infections. It has been demonstrated that HCMV phosphoprotein 70 (pp71) stimulate cell cycle progression by inducing the proteasome-dependent degradation of the retinoblastoma family of tumor supressors.[492] However, pp71 does not induce apoptosis and fails to transform cells, but sends a proliferative signal to cells inducing entry into the cell cycle and progression into S-phase.

The current treatment of HCMV diseases uses nucleoside (acyclovir, ganciclovir) substrate analogues. Toxicity of ganciclovir and emergency of mutants resistant to acyclovir resulted in an intensive research for a new class of antiviral compounds. The X-ray structure of the serine proteases of HCMV and other viruses (HSV-1, HSV-2) revealed that these enzymes belong to a novel class of serine proteases where the active site is composed of the His, His, Ser triad. Design and synthesis of pyrrolidine-5,5′-*trans*-lactames (5-oxo-hexahydropyrrolo-[3,2-*b*]pyrroles,) as novel mechanism based inhibitors of HCMV protease has been reported.[493] The best compounds (Fig. 21) have high human plasma stability with significant *in vitro* antiviral activity against HCMV, and are equivalent in potency to ganciclovir.

A new class of heterocycles, pyrimido[1,2-*b*]-1,2,4,5-tetrazin-6-ones with flavin-like redox properties has been designed and synthesized.[494] These novel inhibitors of HCMV protease show a new mechanism of action: oxidation of several cysteine residues generates cross-linking of the enzyme.

Human rhinoviruses (HRV) are a group of plus-strand RNA viruses belonging to the picornavirus family and represent the leading cause of common cold in humans. HRV 3C protease is a cysteine protease that is responsible for the generation of mature viral proteins, thus, the enzyme is required for viral replication and infections. Due to its essential roles in viral infection and unique protein structure, HRV 3C protease represents an attractive target for the development of antiviral agents. The synthesis and biological evaluation of a series of tripeptidyl α-keto-amides (**94**) as HRV 3C protease inhibitors is described.[495] The most potent inhibitor showed impressive enzyme inhibitory activity as well as antiviral activity against HRV-14. Another research group described the structure-based design, synthesis and biological evaluation of irreversible HRV 3C inhibitors.[496] The optimization of the pharmacokinetic properties of various 2-pyridone containing peptidomimetics was performed and the best compounds proved to be orally bioavailable. The structure of HRV 3C inhibitors is shown in Fig. 22.

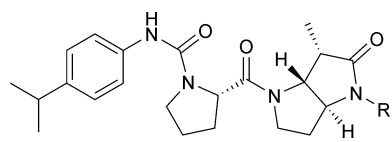


Fig. 21

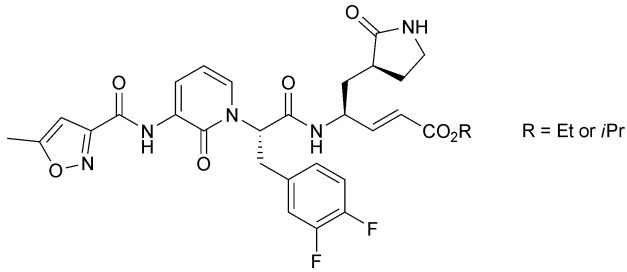
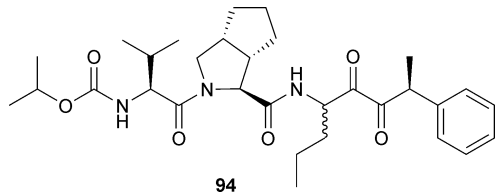


Fig. 22



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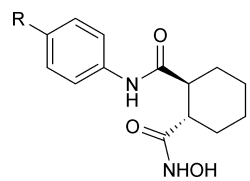
5.7 Converting enzyme (ACE, TACE) inhibitors

Angiotensin-1-converting enzyme (ACE) and its relatives[497] as well as ACE-inhibitors[498] are reviewed. ACE inhibitors have also strong cytostatic properties on cultures of many normal and neoplastic cells *in vitro*, *e.g.* Captopril is effective in controlling tumor growth in a case of Kaposi's sarcoma in humans.[497] The ACE inhibitors containing a thiol group seem to be more effective in controlling fibrosis and the growth of some neoplastic cells than those ACE inhibitors without thiol group in their structure. The blockade of angiotensin II synthesis plays an essential role in the cytostatic activity of these drugs.

Tumor necrosis factor-α (TNF-α) is a cytokine protecting the organism against infection, however, when overproduced, it is proinflammatory in nature. Overproduction of TNF-α has been linked to several diseases including rheumatoid arthritis and Crohn's disease. TNF-α converting enzyme (TACE) is the metalloproteinase that processes pro-TNF-α to the soluble TNF-α. There has been a great deal of interest in the design of TACE inhibitors in order to suppress the release of soluble TNF-α as well as the amount of circulating TNF-α. The beneficial effect of TNF-suppression has been demonstrated clinically *via* two protein-based drugs in rheumatoid arthritis and Crohn's disease. Design, synthesis and evaluation of benzothiadiazepine hydroxamates as selective TACE inhibitors have been described.[498] The best compound was a very active inhibitor of TACE (K_i = 5 nM). A novel series of TACE inhibitors containing a lactam scaffold, *N*-hydroxy-2-(2-oxo-1-pyrrolidinyl)acetamides were designed and synthesized.[499] A structure–activity relationship study indicated that the lactam scaffold binds to TACE and the hydroxamate group binds to the catalytic metal ion in a bidentate fashion. Sulfonate esters of hydroxamic acids are described as potent inhibitors of TACE with excellent selectivity over matrix metalloproteinases MMP-1 and MMP-13.[500]

Rational design based on a broad spectrum of matrix metalloproteinase inhibitors led to the identification of a novel series of cyclic succinate TACE inhibitors (**95**).[501] The best compounds exhibited potent inhibitory activity on porcine TACE and excellent selectivity over metalloproteinases MMP-1, -2 and -9.



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After lead identification of cyclic succinate TACE inhibitors,[501] the modification and optimization led to the identification of a series of piperidine-containing TACE inhibitors with potent activity in the inhibition of TNF-α release.[502] The most potent analogue IM491 [*N*-hydroxy-(5*S*,6*S*)-1-methyl-6-{[4-(2-methyl-4-quinolinylmethoxy)-anilinyl]carbonyl}5-piperidinecarboxamide] exhibited an IC_{50} value of 20 nM with excellent selectivity over MMP-1, -2 and -9 and is orally bioavailable in beagle dogs. TACE inhibitors have been highly sought as potential therapeutic agents not only for the treatment of rheumatoid arthritis but also osteoarthritis. The published literature on these inhibitors from 2001 to 2003 is reviewed.[503] Potent and selective bicyclic heteroaryl hydroxamic acid TACE and metalloproteinase inhibitors were synthesized and the structure–activity relationship was analyzed.[504] Selectivity and efficacy *versus* TACE and MMPs could have been controlled by appropriate substitution on the scaffolds and variation of the sulfonamide P1′ group. The TACE inhibitor drug CP-661, 631 effectively prevents regulated secretion of the amyloid precursor protein (APP) in primary cultures of human neurons but does not influence total Aβ 1–42 levels under these conditions.[505] Novel aza-sugar (L- and D-gulono- and L-gluconotype) based matrix metalloproteinase inhibitors exhibited very potent inhibitory activities against TACE, MMP-1, -3 and -9.[506]

The zinc metalloprotease endothelin converting enzyme (ECE) has been a target for designing inhibitors for potential therapeutic applications in cardiovascular diseases. Synthesis and degradation of endothelin-1 and the role of ECE in the genesis of the most potent pressor peptide, endothelin-1, has been reiewed.[507] ECE proved to be not the sole enzyme in the genesis of endothelins; other enzymes (chymase, MMP-2) have been suggested to be involved in the production of endothelin.

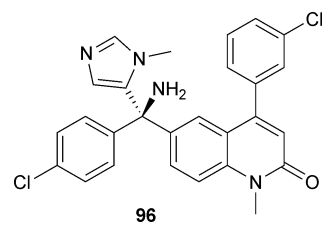
5.8 Elastase inhibitors

Human leukocyte elastase (LE) is a serine proteinase participating in a number of severe acute and chronic pathologies, including inflammation and cancer invasion. The clinical demand for new, more specific and potent inhibitors of LE is growing. The synthesis of a series of 4-alkylidene-azetidin-2-ones (β-lactams) is described[508] as potent leukocyte elastase inhibitors. The novel compounds showed no cytotoxicity against NIH-3T3 murine fibroblasts and are good drug candidates showing selective anti-inflammatory and anti-invasion properties.

Structure–activity relationship of cinnamic acid derivatives as inhibitors of the human neutrophil elastase is reported.[509] Comparison of the IC_{50} values with the results of the ligand docking calculations revealed that the main structural element of the aromatic *ortho*-dihydroxy groups combined with a lipophilic residue seems to be a prerequisite for an optimal binding within the active site of the enzyme.

5.9 Farnesyltransferase inhibitors

Farnesylated Ras-proteins are involved in oncogenesis, thus the inhibition of the enzyme farnesyltransferase has been a very attractive strategy in cancer therapy. 5-Imidazolyl-quinolines,-quinazolinones and -benzoazepinones proved to be potent inhibitors. One of the drug candidates (R115777, Zarnestra™) (**96**) is already in progression to phase III Clinical studies.[510]



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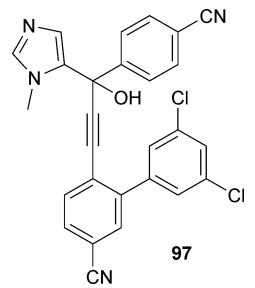
Extensive structure–activity relationship studies for these compounds (modification of R115777) resulted in active compounds, *e.g.* the *in vivo* metabolite *N*-demethylated quinolinone. Replacement of the 1-methyl-imidazol-5-yl moiety in the inhibitor Zarnestra™ series by a 4-methyl-1,2,4-triazol-3-yl group gave us compounds with similar structure–activity relationship profiles, showing that this triazole is potentially a good surrogate to imidazole for farnesyltransferase inhibition.[511] Similarly, substituted azoloquinolines and -quinazolines containing 4-chlorophenyl group demonstrated potent *in vitro* enzymatic inhibition.[512] 4-[3-Biphenyl-1-hydroxy-1-(3-methyl-3*H*-imidazol-4-yl)-prop-2-ynyl]1-yl-benzonitrile (**97**) proved to be a potent, nonpeptidic, non-sulfhydryl, selective inhibitor of farnesyltransferase.[513]



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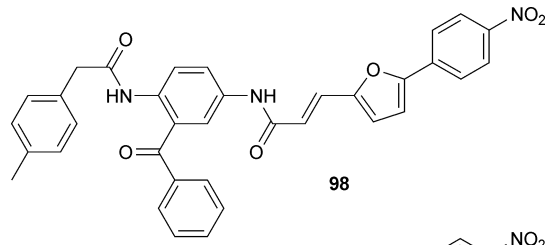
A series of novel analogues of this compound were synthesized and tested *in vitro* for their inhibitory activities. The goal of this research was to identify additional inhibitor molecules having not only good *in vitro* potency but may also have different physical properties, *e.g.* solubility and capability for crystallization. The most promising compound of this series possesses potent enzymatic and cellular activities (4-[(3-methyl-3*H*-imidazol-4-yl)-(2-phenylethynyl-benzyloxy)-methyl]-benzonitrile).[514]

In addition to farnesyltransferase (FT) there are two other protein-isoprenyl-transferases: geranyltransferase type I (GGT-I) and type II (GGT-II). FT and GGT-I act similarly transferring C-15 and C-20 units, GGT-II acts through a different mechanism. Simultaneous inhibition of both FT and GGT-I can lead to toxicity, thus selective FT-inhibitors have been designed as potential anti-cancer agents. A series of pyridine-containing FT-inhibitors has been developed with potent *in vitro* and cellular activity.[515] Some novel non-thiol FT-inhibitors (5-acylamino-benzophenone derivatives) were designed.[516] These compounds turned out to be only weakly active against farnesyltransferase, but displayed an antiproliferative effect rendering them suitable for further development as a novel type of cytostatic agents. The structure of a 2,5-diaminobenzophenone-based FT-inhibitor (**98**) served as lead compound for development of antimalarial drug candidates against multi resistant strains of *Plasmodium falciparum*.[517]

Further non-thiol FT-inhibitors have been designed: the nitrophenylfurylacryl-substituted benzophenone (**99**) served as lead for further optimization.[518] The best compounds have a 2-acylamino substituent at the benzophenone core structure of the initial lead and display activity in the low nanomolar range. A rapid structure–activity study was performed by parallel liquid synthesis on *N*,*N*′-disubstituted 3-amino azepin-2-one to afford potent and specific FT-inhibitors with low nM enzymatic and cellular activities.[519] The activities of the selected compounds were validated *in vivo* and two compounds displayed significant antitumor activity.

Systematic medicinal chemistry studies starting with the lead compound (**100**), discovered from a 5-nitropiperidin-2-one combinatorial library, resulted in a potent series of novel piperidine inhibitors of FT.[520] The best compound inhibited FT in a Ras competitive manner with an IC_{50} of 1.9 nM. A pyridyl moiety was introduced into a previously developed series of FT inhibitors containing imidazole and cyanophenyl,[513,514] resulting in potent inhibitors with improved oral bioavailability.[521] In the same laboratory a series of imidazole-containing biphenyls was



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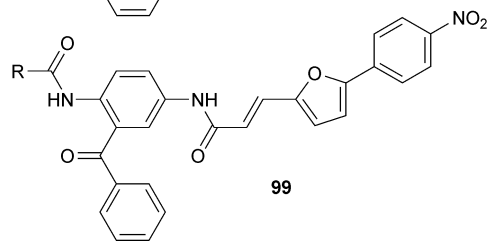

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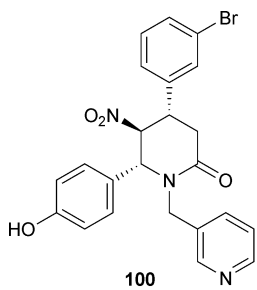
prepared and evaluated *in vitro* for inhibition of farnesyltransferase and cellular Ras processing[522]. Several of these analogues are potent inhibitors of FT (<1 nM) and cellular active (≤ 80 nM).

Aryl tetrahydropyridine compounds (**101**) are potent and selective inhibitors of FT. However, this compound suffers loss of potency in the cellular assay. In an effort to improve on this compound, several other series of tetrahydropyridine containing FT-inhibitors were designed and synthesized.[523] The best novel compounds possess improved cellular potency and bioavailability. Similar aryl tetrahydropyridine compounds containing substituted glycine, phenylalanine or histidine are good inhibitors of farnesyltransferase.[524]



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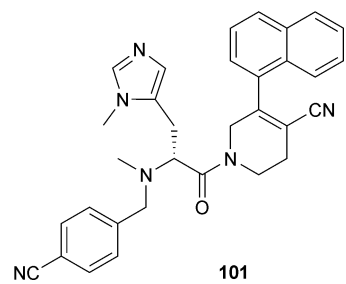

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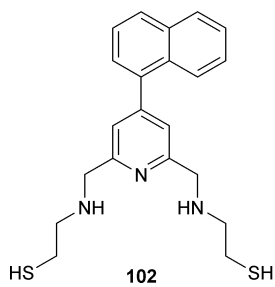
A novel metal chelator comprising a 4-(naphthalene-1-yl) pyridine and 2-aminoethanthiol (**102**) was synthesized.[525] The compound showed inhibitory activity against human FT with an IC_{50} of 1.9 μM. A series of novel diaryl ether lactams (**103**) have been identified as very potent dual inhibitors of protein farnesyltransferase and geranylgeranyltransferase.[526] This compound exhibited excellent activity in both enzyme assay (FTase IC_{50} = 2.9 nM, GGTase IC_{50} = 7.1 nM).



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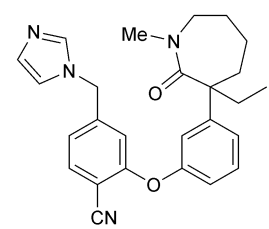

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Some pyrazino[1,2-*a*]indole-1,4-diones (structurally simplified analogues of the natural mycotoxin gliotoxin) have been synthesized and investigated as inhibitors of prenyltransferases.[527] One compound proved to be a selective inhibitor of GGTase I.

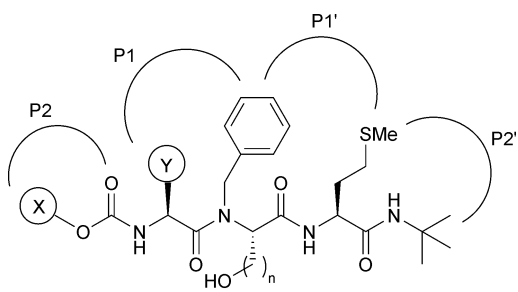
5.10 HIV protease inhibitors

Strategies in the design of prodrugs of anti-HIV agents including nucleoside reverse transcriptase inhibitors and HIV aspartic acid protease inhibitors are reviewed.[430] The latter enzyme remained an attractive target for designing inhibitors for effective antiviral therapy. By 2003, six protease inhibitors have been approved by the FDA for treatment of HIV-1 infection (saquinavir, ritonavir, indiravir, nelfinavir, amprenavir and lopinavir). The high cost of treatment hinder the widespread use of the currently approved HIV-1 protease inhibitors, thus there is a vast need for improved and cost-effective inhibitor drugs. A series of HIV-1 protease inhibitors having new tetrahydrofuran P2/P2′ groups have been synthesized and tested for protease inhibition and antiviral activity.[528] Six novel 4-aminotetrahydrofuran derivatives were prepared starting from commercially available isopropylidene-α-D-xylofuranose.[529] Promising subnanomolar HIV-1 protease inhibitory activities were obtained. Another research group described the synthesis of several new anti-HIV-1 compounds containing a *C*(2) symmetry axis an a dihydroxyethylene moiety based on the D-tartaric acid backbone. The anti-HIV-1 activity was evaluated in PM-1 cells and the values are in micromolar range.

A new strategy was used to overcome the problem of drug resistance: monocyclic HIV protease inhibitors incorporating 15- or 17-membered macrocycle with an equivalent P3 or P3′ groups have been designed and synthesized.[530] The inhibitors contain a unique unnatural amino acid, (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid. The contribution of the macrocycle in the monocyclic inhibitors was important and several inhibitors exhibited low nanomolar inhibitory activity against the wild type HIV/FIV proteases. As a novelty on this research field, rapid diversity-oriented synthesis of HIV protease inhibitors was performed for *in situ* screening in mircotiter plates.[531]

A series of *N*-benzyl pseudopeptides was designed, synthesized and tested as HIV-1 protease inhibitors.[532] (The general structure of *N*-benzyl pseudotripeptides is given in Fig. 23.) The pseudotripeptide Fmoc–Leu–*N*(Bzl)Hse–Met–NH–*t*-Bu was the best inhibitor of the series (IC_{50} = 170 nM) showing promising inhibition of viral replication (ED_{50} = 52 nM). All new compounds exhibit high enzymatic resistance and stability against cell cultures and plasma enzymes.

A highly flexible method was extended for rapidly assembling aspartic protease inhibitors to produce symmetric and asymmetric monohydroxyethylene peptidomimetics.[533] The novel peptidomimetics inhibit both HIV-1 and *Candida albicans* aspartic proteases. A computational approach was applied in which the strength of an HIV-protease inhibitor is determined from its interaction energy with a limited set

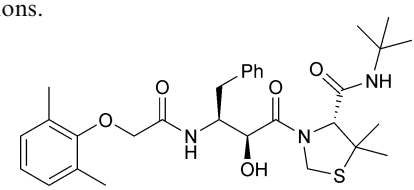


X = Fmoc, Bzl; Y = Bzl, *i*Bu; n = 1, 2

Fig. 23

of amino acid residues of the inhibited protein.[534] The method uses a consensus structure of HIV protease built from X-ray crystallographic data and all inhibitors are docked into the consensus structure. This approach provides insight in which interactions are important for inhibiting HIV-protease and it allows for quantitative prediction for inhibitor strength. The synthesis and structure–activity relationship of HIV-protease inhibitors derived from carbohydrate alditols were discussed.[535] A new series of 1,2,5,6-tetra-*O*-benzyl-D-mannitol derivatives exhibited submicromolar activity against HIV-protease. These compounds contain no nitrogen and are readily prepared in a few chemical steps from inexpensive starting materials.

KNI-727 (**104**) is a sparingly water soluble HIV-1 protease inhibitor. A series of water soluble prodrugs was designed and synthesized, all prodrugs contain a water-soluble auxiliary with tandem linked units *i.e.* self-cleavable spacer and a solubilizing moiety with an ionized amino function.[536] This structure exhibits a marked increase in water solubility ($>10^4$-fold; KNI-727 has a water solubility of 5.5 μg/mL). All the water soluble prodrugs tested regenerated the parent drug KNI-727 *in vitro* as well as *in vivo* and showed good bioavailability (23 to 29%; 1.5–1.9-fold higher than that in the administration of the parent drug alone. The structures of the prodrugs are based on O → N intramolecular acyl migration reaction under mild basic conditions (pH 7.4),[537] thus these prodrugs were rapidly converted to the parent drug under physiological conditions.



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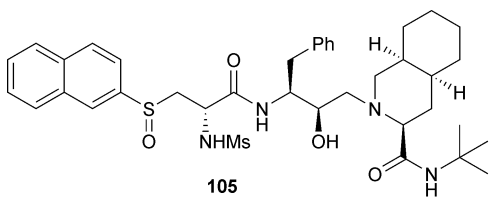
Atazanavir is an azapeptide HIV-1 protease inhibitor and is currently in phase III clinical trials. The use of highly efficient Lewis acid–Lewis base bifunctional asymmetric catalysts was reported for the asymmetric synthesis of chiral building blocks of Atazanavir.[538]

Novel HIV-protease inhibitors containing a hydroxyethylamine dipeptide isostere as a transition-state-mimicking structure were synthesized by combining substructures of known HIV-protease inhibitors saquinavir, nelfinavir, amprenavir and Lilly's compound.[539] Some of them, TYA5 (**105**) and TYB5 (**106**) were proven to be not only potent enzyme inhibitors (K_i = 0.12 nM and 0.10 nM, respectively) but also strong anti-HIV agents (IC_{50} = 9.5 nM and 66 nM, respectively).



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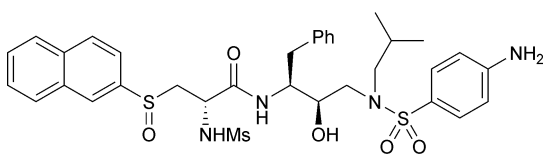

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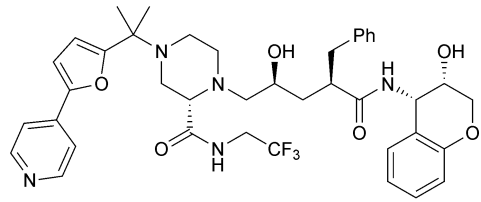
Several HIV-protease inhibitors contain hydroxyethylurea isosteres. It has been reported that the replacement of *t*-butylurea moiety by benzothiazolesulfonamide provided inhibitors with improved potency and antiviral activities.[540] Another

research laboratory described the design and synthesis of a series of highly potent HIV protease inhibitors, the new structure is a hybrid of indinavir and JE-2147.[541] The novel compounds are active against various clinical viral isolates as well as wild-type virus. The same research laboratory reported the design and synthesis of novel HIV-protease inhibitors with picomolar potency against inhibitor-resistant HIV-1 strains.[542] The novel compounds contain biaryl pyridylfuran P_3 substituent on the hydroxyethylene isostere scaffold. The best compound (**107**) (containing a *gem*-dimethyl substituent) showed 100% oral bioavailability (dogs) and more than doubled the $t_{1/2}$ of indinavir. The same research group described novel HIV-1 protease inhibitors active against multiple inhibitor-resistant viral strains.[543] The novel inhibitors contain an *N*-arylpyrrole moiety in the P_3 position and their oral coadministration with indinavir hindered the metabolism by the cytochrom P450 3A4 isoenzyme.



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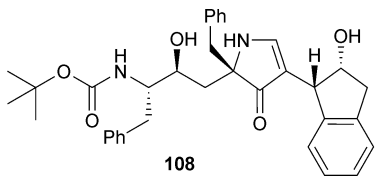
The design, synthesis and biological evaluation of a series of HIV-1 protease inhibitors based upon the 3,5,5-trisubstituted pyrrolin-4-one scaffold is described.[544] Compounds (**108**) and (**109**) are two active members of this series. Novel potent HIV-1 protease inhibitors based on the structure of Ritonavir were synthesized for maintaining high blood concentrations and possessing high activity against resistant viruses.[545]



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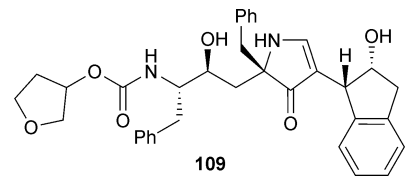

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5.11 Matrix metalloproteinase inhibitors

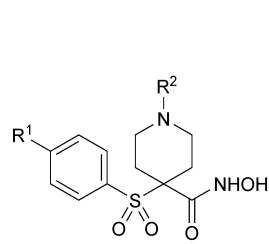
Abnormal production of matrix metalloproteinases (MMPs), a group of tightly regulated metalloproteases, has been observed in a variety of diseases, such as emphysema, atherosclerosis, and cancer metastasis. TNF-α converting enzyme (TACE) is also a metalloproteinase, its inhibitors were already summarized in Section 5.7.[498–506] MMPs are preferred target proteins for inhibitor design. Destruction of connective tissue ensues and elastin is often a key target. Three of the main elastolytic MMPs are the following: gelatinases MMP-2 and -9 and the metalloelastase MMP-12. To investigate the possibility of peptides to inhibit the elastolytic activity of these enzymes the mapping of the recognizing sites for MMPs within tropoelastin was performed.[546] Peptides that correspond to regions overlapping these sites were then tested for their ability to inhibit these MMPs. The presence of a free sulfhydryl or hydroxamate group capable of chelating the zinc ion in the active site of the MMPs was generally found to increase the inhibitory activity of the peptides. K_i values for the inhibitors were in micromolar range.

Previously a series of bisarylthioethers as MMP inhibitors was published, more recently the synthesis and structure–activity relationship studies of a series of 5-substituted 2-bisarylthiocyclopentane carboxylic acid as MMP inhibitors were described.[547] Potent and specific MMP-2, -3, -9 and -13 inhibitors were obtained by region- and stereoselective substitutions at positions 2 and 5 on the cyclopentane

ring. Two novel compounds were active in the mouse B16-F10 metastasis model and display very good pharmacokinetic parameters.

A series of novel and orally active *N*-substituted 4-benzenesulfonylpiperidine-4-carboxylic acid hydroxyamide derivatives (**110**) have been synthesized.[548,549] One of the compounds turned out to be a potent, selective and an orally active MMP inhibitor in the clinically relevant advanced rabbit osteoarthritis model.

The overexpression of MMP-3 and MMP-13 is associated with nonhealing wounds, whereas active MMP-1, -2, -9 and -14 are required for normal wound healing to occur. The synthesis and enzyme inhibition profile of a novel MMP inhibitory compound KK-370, 106 (**111**) were described.[550] The results of biological tests suggested that KK-370, 106 was sufficiently potent to inhibit MMP-3 mediated matrix degradation while did not affect cellular migration mediated by MMP-1, -2 and -9. These properties make compound KK-370, 106 a suitable candidate for progression to clinical trials in human chronic dermal wounds, such as venous ulcers.



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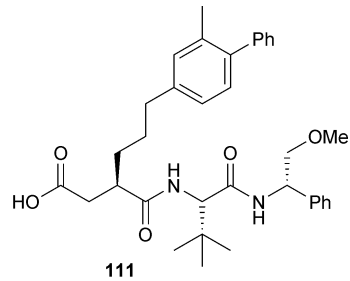
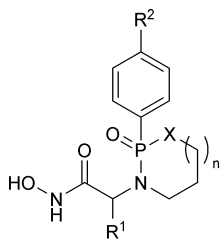


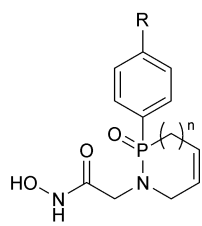
111

In order to investigate structure–activity relationships of azasugar series toward metalloproteins, a series of azasugar based compounds was synthesized.[506,551] The 4-phenoxybenzene derivative of the azasugar arysulfonylamide exhibited the most potent inhibitory activities against MMP-1, -3, -9 and TACE.

The design, synthesis, and structure–activity relationship of a series of novel nonpeptidic cyclic phosphon- and phosphinamide-based hydroxamic acids as inhibitors of matrix metalloproteinases MMP-1, -3 and -9 (**112–114**) were described.[552]



112 X = O
113 X = CH_2



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Based on modelling studies and X-ray analysis, a model of the binding mode of these novel compounds in the MMP active site was obtained. Another research group reported the synthesis of a series of novel MMP/bacterial collagenase inhibitors incorporating arylsulfonylureido and 5-dibenzosuberenyl/suberyl moieties.[553] These new compounds were assayed as inhibitors of MMP-1, -2, -8 and -9 as well as of the collagenase isolated from *Clostridium histolyticum* (ChC). Several of these inhibitors also showed selectivity for the deep pocket enzymes (MMP-2, -8 and -9) over the shallow pocket ones MMP-1 and ChC.

5.12 NO-synthase inhibitors

Three distinct human isoforms of nitric oxide synthase (NOS) have been identified: the neuronal (nNOS), the endothelial (eNOS) and inducible enzyme (iNOS). The

enzyme contains a heme group and produces NO by conversion of L-arginine to L-citrulline by a five-electron oxidation reaction. Selective inhibitors of the isoforms of NOS are drug candidates in the treatment of certain diseases arising from the overproduction of NO by NOS enzymes. The role of NO in angiogenesis has been reviewed.[11] Two reviews exhibit the emerging role of asymmetric dimethylarginine (ADMA) as a cardiovascular risk factor.[554,555] Elevated plasma concentration of ADMA induces dysfunction of the endothelium, thus, ADMA is becoming a good target for pharmacotherapeutic intervention.

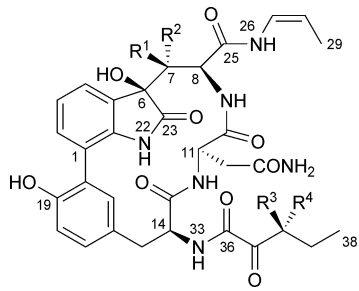
Activated microglia extensively produces NO by inducing expression of inducible NO synthase. A series of styrylheterocycles was prepared and their inhibitory activities against NO-production were evaluated in a cell culture system.[556] structure–activity relationship studies suggest that the suppression of iNOS mRNA transcription is related to the inhibitory activity of styrylheterocycles. 1,3-Selenazol-4-one derivatives were described as inhibitors of iNOS-mediated NO production in lipopolysaccharide (LPS) induced BV-2 cells, a murine microglia cell line.[557] Some chalcones exert potent anti-inflammatory activities by inhibiting NO production in LPS/interferon γ activated N9 microglial cells. It was demonstrated that most of the 2′,5′-dihydroxychalcones have anti-inflammatory effects, probably through the suppression of iNOS protein expression, thus these chalcones may be useful for the relief of septic shock.[558] A novel homologue of thiocitrullin (*e.g.* N^{ε}-(4,5-dihydrothiazol-2-yl)-L-lysine) was reported as potent and non-isoform selective inhibitor of NOS.[559]

The same research group performed the synthesis of *N*-benzyl- and *N*-phenyl-2-amino-4,5-dihydrothiazoles and thioureas as potential modulators of the isoforms of NOS.[560] The discovery of a novel class of NOS inhibitors, 2-substituted 1,2-dihydro-4-quinazolinamines and the related 4′-aminospiro[piperidine-4,2′(1*H*)-quinazolin]-4-amines is described.[561] Members of both series exhibit nanomolar potency and high selectivity for iNOS. Design and synthesis of orally bioavailable inhibitors of *i*NOS, including fused bicyclic compounds,[562] α-, β-unsaturated cyclic amidines (dihydropyridin-2(1*H*)-imines and 1,5,6,7-tetrahydro-2*H*-azepin-2-imines)[563] as well as 2-azabicyclo[4.1.0]heptan-3-imines[564] were reported. The synthesis of 4-fluorinated L-lysine analogues as selective iNOS inhibitors was performed and their inhibitory activity was demonstrated.[565]

Preservation of physiologically important NO functions requires the use of isoform-selective inhibitors of NOS enzymes. Design and synthesis of aromatic peptidomimetics containing reduced amide bond as potential selective nNOS inhibitors were reported,[566] but their selectivity for nNOS has not yet reached the optimal level. Another research group performed the synthesis of dihydroquinoline compounds with aminoalkyl side chain[567] as potent and selective nNOS inhibitors. A marked selectivity *versus* endothelial NOS of up to approximately 300-fold was observed, whereas iNOS was moderately inhibited. The synthesis, pharmacological evaluation and modeling of 7-methoxyindazole and related alkoxy-indazoles as novel inhibitors of nNOS were presented.[568]

5.13 Proteasome inhibitors

Proteasomes (large cytosolic protease complexes) participate in regulation of diverse intracellular processes, including degradation of cytosolic proteins in the cell and inflammatory response. A special review deals with the interruption of tumour cell cycle progression through proteasoma inhibitors.[433] Proteasome inhibitors are mostly short oligopeptide sequences or peptidomimetics. A series of new 3-alkoxy-7-amino-4-chloroisocoumarin derivatives were synthesized and evaluated as inhibitors of 2OS proteasome and other representative classes of proteases (Ser-proteases, Cys-proteases, Asp-proteases).[569] The novel compounds show only weak or moderate inhibitory activity on the 2OS proteasome but prevent γ-secretase-mediated production of Aβ 1–40 and 1–42 peptides and thus can be considered as possible hits

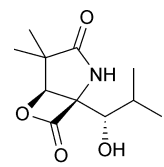


TMC-95	R^1	R^2	R^3	R^4
A	H	OH	CH_3	H
B	H	OH	H	CH_3
C	OH	H	CH_3	H
D	OH	H	H	CH_3

Fig. 24

for the development of novel agents for preventing Alzheimer's disease. Natural cyclic peptides TMC 95A and B represent a new class of noncovalent, selective proteasome inhibitors (Fig. 24).[570] To explore the structure–activity relationship of this class of inhibitors, a series of TMC 95A/B analogues were synthesized and analyzed. The novel analogues with simplified structure (alkylamide functionality instead of enamide structure) retain the potency of proteasome inhibitory activity, thus the more accessible simple analogues could serve as potential therapeutic agents.

A short, stereocontrolled and practical synthesis of α-methylmuralide (**115**), a selective inhibitor of proteasomes has been developed.[571] The new synthetic route gives high overall yield and the scale up to the synthesis is easy. Proteasome inhibitors (*e.g.* MG132, PSI, PSII and III) represent a novel tool to suppress human cytomegalovirus (HCMV) replication and virus-induced immune modulation.[572] The experimental data suggest that short-term therapy with proteasome inhibitors might be an alternative strategy to prevent inflammatory diseases caused by HCMV.



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5.14 Protein phosphatase inhibitors

Protein kinases and phosphatases are classes of enzymes that are becoming increasingly important as targets for drug discovery. Protein phosphorylation and dephosphorylation is the key regulatory and mechanism for most essential cellular functions, including gene transcription, cell growth, cell metabolism, and immune response. The balance between phosphorylated and dephosphorylated proteins, which is controlled by protein kinase and phosphatase activities, is crucial for maintaining proper cellular function. Protein phosphatases 1 and 2A (PP1 and PP2A) represent two of the four major classes of serine–threonine phosphatases, which also include PP2B (calcineurin), PP2C, and others.

Analogues of the potent and moderately selective PP1/PP2A inhibitor tautomycin (TM) were prepared with modification of the C1′–C7′ anhydride moiety, activity measurements demonstrated that the anhydride moiety is not critical in controlling the selectivity of inhibition.[573] A series of phosphonothioic acids and corresponding phosphonic acids were synthesized and their inhibitory properties were compared toward different phosphatases.[574] Despite different steric requirements and differences in charge distribution in the anions of phosphonothioic acids compared to

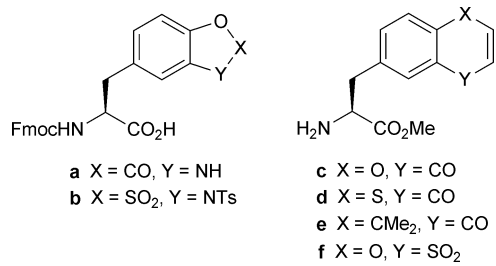


Fig. 25

phosphonic acids, it was found that the differences in inhibitory properties were modest.

A series of acyclic, truncated microcystin analogues, comprised of the dienic β-amino acid and up to four additional amino acids characteristic of the parent toxin, was synthesized and screened for activity as inhibitors of PP1 and PP2A.[575] None of the truncated products approached the potency of microcystin itself. Analogues of another antibioticum cytostatin (a serine/threonine phosphatase 2A inhibitor) were synthesized and evaluated as inhibitors of various phosphatases.[576]

PP2B (calcineurin) plays an important role in intracellular signal transductions. The immunosuppressants FK506 and cyclosporine A bind to immunophilins, and these complexes selectively inhibit PP2B. It is of interest to find a direct and selective inhibitor of PP2B that does not involve the immunophilins as a biological tool for studies of PP2B and as a drug candidate. Cantharidin served as lead compound for design and synthesis of several derivatives.[577] Among these compounds, 1,5-dibenzoyloxymethyl substituted norcantharidin proved to be a very highly selective catalytic site-directed inhibitor of PP2B.

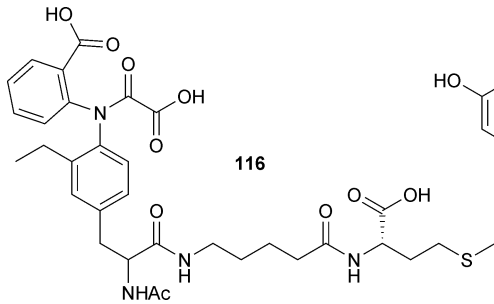
Protein tyrosine phosphatases (PTPases) are enzymes that regulate signal transduction pathway *via* dephosphorylation of biologically important proteins. Small molecule PTP1B inhibitors have considerable therapeutic potential for the treatment of Type 2 diabetes and obesity. For these purposes a novel series of orally active pyrimido[5,4-3][1,2,4] triazine-5,7-diamine-based hypoglycemic agents have been identified[578] displaying oral glucose lowering effects in *ob*/*ob* mice. However their inhibitory properties against PTP1B is not selective. Some novel analogues of the well known PTPase inhibitor dephostatin were synthesized and studied in biological experiments. The methoxine- and hexyl-methoxine-3,4-dephostatin analogues showed antidiabetic activity *in vivo*.[579] Several phenylalanine derivatives containing unusual amino acid designed as phosphotyrosine mimetics or irreversible active site inhibitors were synthesized (Fig. 25).[580]

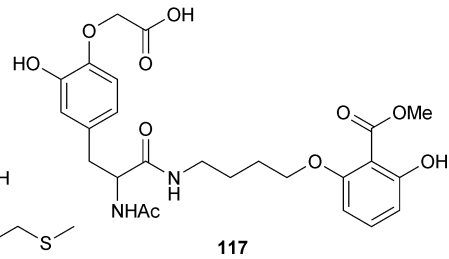
Many PTP inhibitors are peptide based and contain a highly charged phosphate-mimicking component causing each of membrane permeability. Employing a non-charged phosphate-mimic and non-peptidyl structural components a novel series of trifluoromethyl sulfonyl and trifluoromethyl sulfonamide compounds as PTP inhibitors were designed and synthesized.[581] The novel compounds of uncharged phosphate mimic display general, reversible and substrate-competitive inhibition of PTPs.

One common approach for designing PTP inhibitors has been to incorporate a nonhydrolysable phosphotyrosine (pTyr) mimic into a peptide substrate of PTPs. The synthesis of three such nonhydrolyzable pTyr mimics was described. The potential PTP inhibitors contain α-ketoacid, α-hydroxyacid, and methylensulfonamide functional groups in place of the phosphate.[61] These pTyr mimics were built into the peptide sequence Ac–Asp–Ala–Asp–Glu–X–Leu–NH_2, where X is the pTyr mimic, and analyzed for inhibitory activity against PTPs. Another research group reported the design and synthesis of tripeptide inhibitors against PTP of *Yersinia*

pestis.[582] The mono-anionic peptide, Fmoc–Glu(OBzl)–Xxx–Leu–amide (Xxx = 4-(carboxymethyloxy)Phe) provided an IC_{50} value of 2.8 μM against Yersinia PTP.

Previously a novel series of oxalyl-arylamino benzoic acid based, catalytic-site directed, competitive, reversible PTP1B inhibitors was reported. Using a solution phase parallel synthesis approach (library) a highly potent PTP1B inhibitor (K_i = 76 nM) (**116**) was identified.[583] The same research group identified a monoacid-based, cell permeable, selective inhibitor of PTP1B.[584] A 2-hydroxyphenoxyacetic acid based phosphotyrosyl mimetic has been linked with an optimized second arylphosphate binding site ligand to produce the best compound (**117**) with low micromolar potancy against PTP1B.





PTP1B is a key negative regulator of both insulin and leptin signaling pathways. Oxalylarylaminobenzoic acid derivatives or reversible, selective and competitive PTP1B inhibitors lower the plasma glucose level *in vivo* in *ob*/*ob* mice.[585] The same research group reported that salicylic acid based ligands bind to the second phophotyrosine binding site of PTP1B, the screening of novel compounds was performed by NMR methods (NMR-based linked fragment screening).[586] Using the same screening methods plus additional X-ray crystal structure-based assembly, a small molecule inhibitor of PTP1B was found with low micromolar inhibition constant and good cellular activity in COS-7 cells.[587,590]

Formylchromone derivatives represent a novel class of PTP1B inhibitors.[588] The most potent inhibitor showed an IC_{50} of 4.3 μM against PTP1B and strong or medium selectivity against other human protein tyrosine phosphatases. (This compound, however, is not selective against microbial PTP-ases). Further improvements of the formylchromone derivatives provide a novel pharmacophore for the design of drugs for the treatment of type 2 diabetes and obesity. Theoretical chemical methods (molecular mechanics-based empirical free energy function for compound potency prediction) were used to understand the driving forces in the binding of small molecule inhibitors to the active site of PTP1B.[589] A set of compounds with known activities was docked into the active site, the related energy components and molecular surface areas were calculated.

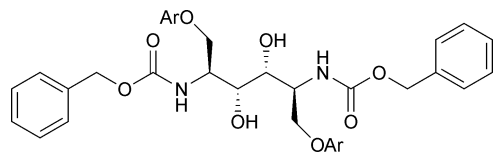
Cdc25a and Cdc25b phosphatases are important cell cycle regulators, overexpression of these phosphatases correlates with a wide variety of cancer, making the Cdc25 enzymes attractive drug targets for anticancer therapies. A new class of Cdc25 inhibitors was reported: indolydihydroxy quinones bind reversibly to the active site of Cdc25s with submicromolar potency.[591] Structure–activity relationships in the 50 derivatives of the lead molecule 2,5-dihydroxy-3-(1*H*-indol-3-yl)[1,4]benzoquinone show interesting and consistent trends for inhibition all three isoforms of Cdc25. The indolyldihydroxyquinones compete effectively with the protein substrate for Cdc25 *in vitro* and lead to rapid cell death *in vivo*. The synthesis of bioactive sesterterpenoid γ-hydroxy butenolides was reported; the novel compounds were inhibitors of Cdc25a and b and showed an IC_{50} of ~2 μM by inhibiting cellular proliferation in a number of human leukaemic and solid tumor cell lines.[592] Ditopic dynamic combinatorial libraries of bis-cationic heterocycles were generated and screening

toward inhibition of the bifunctional enzyme HPr kinase/phosphatase from *Bacillus subtilis*.[593]

5.15 Renin and other aspartyl protease inhibitors

Most of the development programs of renin inhibitors have been closed. The development and evaluation of a potent non-peptidic renin inhibitor, aliskiren, has been reviewed.[594] Aliskiren has reasonable oral bioavailability with practically low level of adverse effect, because of high specificity of renin for only one substrate, namely angiotensinogen. Aliskiren has been shown to effectively reduce angiotensin-II levels in normal volunteers and to lower blood pressure in patients with mild to moderate hypertension.

Recently aspartyl proteases have emerged as new and promising targets for antimalarials. In one of the stages of Plasmodium rely on blood cell hemoglobin for nutrient supply. Two homologues aspartic proteases, plasmepsin I and II (Plm I and Plm II) appear to initiate this hemoglobin degradation process in the parasite and blockade of these enzymes has been demonstrated to result in parasite death. The drug discovery efforts for designing plasmepsin II inhibitors are reviewed.[595] A series of malaria Plm I and II inhibitors containing a C_2-symmetric core structure (**118**) have been synthesized and tested for protease inhibition activity.[596,597] These compounds can be prepared using a straightforward synthesis involving a phenol nucleophilic ring opening of a diepoxide. The best inhibitors acted both on Plm I and II with K_i values between 0.25 and 2.7 nM as well as showed more than 100-fold selectivity against cathepsin D. The newest compounds of the same research group exhibited picomolar to nanomolar inhibition constant for the plasmepsins and no measurable affinity to the human enzyme cathepsyn D.[598–600]



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Sets of compound libraries have been synthesized from novel reversed-statine isosteres, using a combination of solution phase and solid phase chemistry.[601] The products were evaluated for their Plm I and II inhibiting properties and were found to have modest activity. The same research group described the synthesis of reversed statine type inhibitors of Plm I and II, many of which are azo-peptides.[602] The best inhibitor exhibits K_i values of 250 nM an 1.4 μM for Plm I and II, respectively.

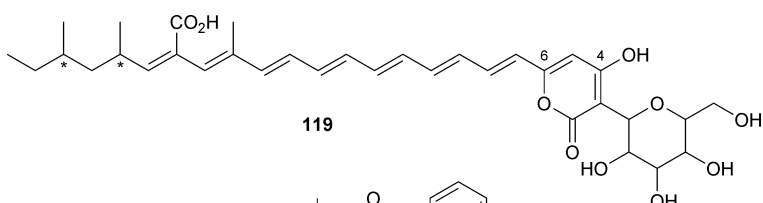
Proteolysis of the amyloid precursor protein (APP) with β- and γ-secretases generates toxic β-amyloid peptides participating in the progression of Alzheimer's disease. The above mentioned aspartyl proteases are preferred target enzymes of the inhibitor design for the treatment of Alzheimer's disease. β-Secretase (BACE) is an integral membrane protease and a high molecular weight complex of the enzyme is more active than the monomer.[603] The BACE complex is enriched in the lipid raft fraction prepared from brain membranes. BACE-expressing human cells are suitable for *in vitro* screening of putative BACE inhibitors.[604] A series of statine-derived sequences were identified that dose-dependently inhibited BACE activity with IC_{50} in micromolar range. The hydroxyethylene transition state isostere was developed as a scaffold to provide potent, small molecule inhibitors of BACE, the most potent compound with the N-terminal isophthalamide proved to be a good inhibitor toward the enzyme.[605] Statine based tetrapeptide BACE inhibitors were designed and synthesized using a heptapeptide BACE transition-state mimetic as the starting point, the best novel compounds possessed an IC_{50} value <100 nM.[606] Human β-secretase and BACE-inhibitors are reviewed.[607] Several BACE pseudopeptide type inhibitors were designed based on hydroxyethylamine dipeptide isostere structures

(IC_{50} <100 nM).[608] A series of novel 7-substituted-4-chloro-3-alkoxy isocoumarin derivatives were synthesized and evaluated as inhibitors of different proteases. Some of the compounds can be considered as possible hits for the development of new agents directed towards Alzheimer's disease.[609]

5.16 Telomerase inhibitors

Human telomerase is a reverse transcriptase that is expressed in essentially all cancer cells, but not in the vast majority of normal somatic cells. The ends of chromosomes (telomeres) are subject to progressive shortening in normal somatic cells, leading ultimately to irreversible growth arrest. In contrast, telomeres in all cancer cells are stabilized in length and effectively immortalized by the enzyme telomerase, which catalyzes the synthesis of telomeric DNA repeats. Several strategies have been devised for the inhibition of telomerase in the hope that this will results in anticancer effects. These methods are reviewed and critically evaluated for their potential in anticancer therapy.[610,611] The catalytic subunit of telomerase is the telomerase reverse transcriptase (TERT), is regulated by interaction with the 90 kDa heat shock protein (HSP 90) and by Akt-dependent phosphorylation. It was demonstrated that HSP90 and Akt physically interact with TERT,[612] this association is necessary for maintaining telomerase activity and inhibition of apoptosis. The hypothesis that simultaneous shortening of the telomers and inhibition of telomerase results in synergistic and tumor-selective cytotoxicity was evaluated and demonstrated that combined use of agents targeting both telomere and telomerase yielded synergistic activity.[613] It was reported that the endogenous chromatin environment plays a critical role in the regulation of telomerase reverse transcriptase (TERT) expression during cellular immortalization.[614] BRCA 1, a gene encodes a 1863-amino acid, 220 kDa nuclear phosphoprotein and acts in concert with DNA-repair enzymes to maintain the integrity of the genome. It was demonstrated that BRCA1 was involved in regulating cellular immortalization on the TERT promoter.[615] The knowledge on timing for the DNA synthesis and circadian telomerase activity provides a model for exploring optimal timing of chromotherapy for treatment with telomerase inhibitors.[616]

Synthetic studies directed toward the assembly of the *C*-glycoside fragment of the telomerase inhibitor D8646-2-6 was reported.[617] The drug candidate (**119**) was isolated as a telomerase inhibitor from the culture broth of *Epicoccum purpurescens*. This is a first example of the *C*-glycosylation using electron-pure aromatics, 4-hydroxypyrone, as a glycosyl receptor. The glycosylation reaction and base-promoted isomerization affords desired β-*C*-glycoside in a 61% overall yield. A small molecule aromatic compound, BIBR 1532 (**120**) has been reported to be a potent telomerase inhibitor. It causes telomerases to shorten and reduces tumor cell proliferation, suggesting it was a lead for the development of anti-telomerase therapy. The synthesis and the evaluation of their ability to inhibit telomerase of BIBR 1532 and derivatives was described.[618]



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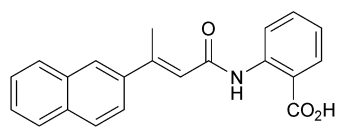

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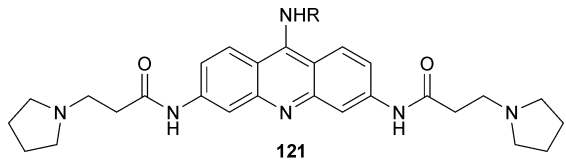
Synthesis of a series of 6-formyl-pyridine-2-carboxylate derivatives and their telomerase inhibitory activities are described.[619] Palladium(0)-mediated syntheses of quino [4,3,2-κI]acridines bearing peripheral substituents as potential telomere maintenance inhibitors is described.[620] The synthesis and evaluation for telomerase-inhibitory and quadruplex DNA binding properties of three related series of rationally designed trisubstituted acridine derivatives (**121–123**) are reported.[621] These are substituted on the acridine ring at the 2, 6, 9 and 3, 6, 9 positions. Molecular modeling calculations support a model for the action of these compounds that involves the stabilization of intermediate quardruplex structures inhibiting the elongation of telomeric DNA by telomerase in tumor cells.



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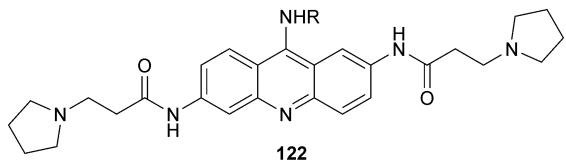

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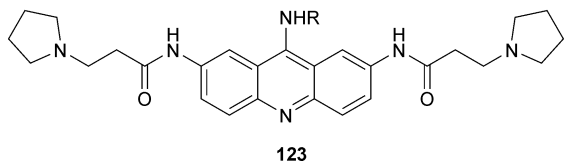

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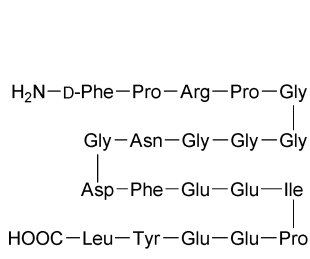
A series of 1,5-bisanthraquinones and 1,5-bisacyloxyanthraquinones was synthesized and their effect was evaluated on telomerase activity and telomerase expression[622] using the cell-based reporter system. In another experiment a series of metalloporphirins (porphirin–aminoquinoline conjugates) was prepared in order to target the G-quadruplex structure of telomeric DNA for the design of antitelomerase compounds.[623] All porphirin complexes were capable of inhibiting the telomerase enzyme with IC_{50} values of micromolar range.

A short review summarises the importance of guanine-rich repetitive sequences, the formation of a quadruplex structure at the 3′-end of telomeric DNA and stabilizing the quadruplex formation resulting in telomerase inhibition.[624] Implications for antitumor therapy with such molecules, the particular challenges and problems are discussed.

A series of oligonucleotide N3′ → P5′ thio-phosphoramidates as templates of telomerase antagonists was designed and evaluated.[625] Novel short oligonucleotide conjugates as inhibitors of human telomerase were designed and synthesized.[626] These compounds contain a relatively short (6–7-mer) oligonucleotide domain, with an N3′ → P5′ phosphoramidate or thio-phosphoramidate backbone. The most potent compounds in this series inhibited telomerase with low nanomolar IC_{50} values in biochemical assays. Sugar-modified nucleotide analogs (arabinofuranosyl-guanine-5′-triphosphate; 3′-azido-2′,3′-dideoxyguanosine-5′-triphosphate) and their thymine counterparts were synthesized and investigated.[627] Triphosphate derivatives of biologically active nucleotides showed telomerase inhibitory effect (K_i values in micromolar range).[628]

5.17 Thrombin and factor Xa inhibitors

Most of the big drug development programs of thrombin inhibitors have been closed. The newly-developed category of anti-thrombotic drugs, the direct thrombin inhibitors are reviewed.[629] These agents interact with thrombin and block its catalytic activity on fibrinogen, platelets and other substrates. Low molecular weight peptidomimetic thrombin inhibitors (bivalirudin (**124**), argatobran (**125**), ximelagatran (**126**) and dabigatran (**127**)) are reviewed.[630]



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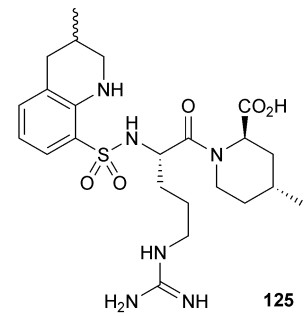

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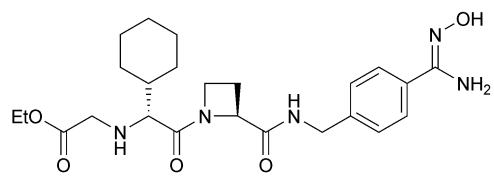

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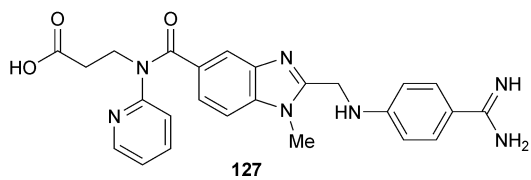

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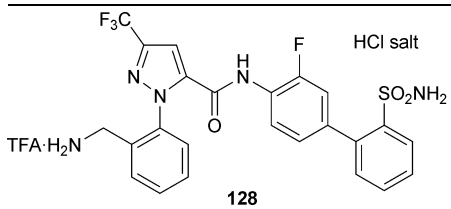
Factor Xa is a trypsin-like serine protease positioned at the convergence of the intrinsic and extrinsic blood coagulation pathways. A series of benzoxazinone derivatives was designed as synthesized as factor Xa inhibitors.[631] The same research group designed and synthesized novel unsubstituted piperazinone-based transition state inhibitors.[632] The new compounds were very potent inhibitors against factor Xa (after optimization IC_{50} values are below 1 nM) and selective over thrombin. Quinoxalinone derivatives as prototypes of dual thrombin and factor Xa inhibitors have been discovered.[633] Nanomolar inhibition of both coagulation enzyme resulted in very potent antithrombotic activity *in vitro*. A potent, selective and orally bioavailable inhibitor of factor Xa, 1-(2-aminomethylphenyl)-3-trifluoromethyl-*N*-[3-fluoro-2′-(aminosulfonyl)[1, 1′-biphenyl)]-4-yl]-14-pyrazole-S-carboxyamide (DPC602) (**128**) was discovered.[634]

An efficient four-step synthesis of 1-aryl-carbamoyl-2-alkyl-4-aryl-semicarbazides starting from benzophenone hydrazone is described leading to moderately active neutral factor Xa inhibitors.[635] Structure-based design of novel guamidine/benzamidine mimics led to the discovery of a potent and bioavailable factor Xa inhibitor SQ311 (**129**) as novel anticoagulant.[636]

Highly constrained factor Xa inhibitors were designed and synthesized, the novel compounds are amidine-substituted bis(benzoyl)-[1,3]-diazepan-2-ones and bis(benzylidene)-bis(*gem*-dimethyl)cycloketones.[637] A novel isonitrile derivative was



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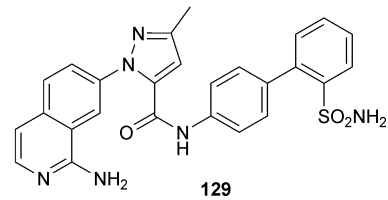

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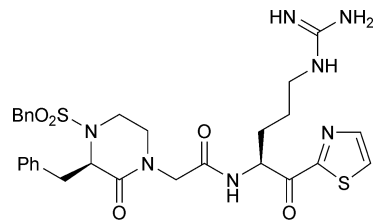
synthesized in an Ugi four-component coupling reaction to explore aryl group substitution effects on inhibition of the coagulation cascade serine protease factor Xa.[638] A series of 2-substituted-4-amidinophenyl-pyruvic and -propionic acid derivatives were designed, synthesized and evaluated as factor Xa inhibitors.[639] The structure–activity relationship of a novel series of substituted piperazinone-based factor Xa inhibitors was described, the most potent compound (**130**) displayed IC_{50} of 0.9 nM.[640]



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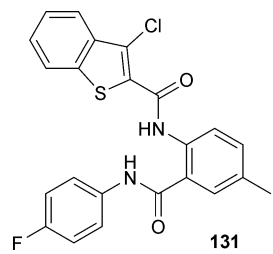
The discovery and structure–activity-relationship of ketopiperazino methylazaindoles as factor Xa inhibitors are described.[641] High-throughput screening gave a novel, potent and non-amidine factor Xa inhibitor (**131**) with good selectivity against thrombin and trypsin.[642] Optimization of the lead structure was performed by modifying the three aromatic groups, substitution of fluorine to chlorine or bromine led to the discovery of subnanomolar factor Xa inhibitors. A series of tetrazole compounds as factor Xa inhibitors was described containing benzamidine mimics as the P_1 substrate, of which the aminobenzisoxazole moiety was found to be the most potent benzamidine mimic. SR374 (**132**) inhibits factor Xa with a K_i value of 0.35 nM and is very selective over thrombin and trypsin.[643]



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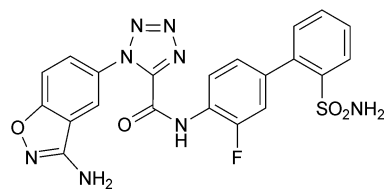

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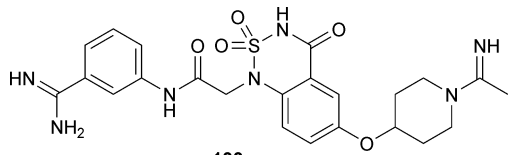
The structure of the known factor Xa inhibitor YM-60828 was optimized, the most effective anticoagulant YM-169920 (**133**) was selected for further *in vivo* anticoagulant studies.[644] The oral bioavailability of fluoro acrylamides, novel cyclic diimide amidine factor Xa compounds, was improved by replacing of the amidine group with methyl amidrazone.[645]

Serine protease VIIa in circulating blood forms the tissue factor/VIIa (TF/VIIa) complex. There is evidence from a lot of research groups that selective inhibition of the TF/VIIa complex may provide effective anticoagulation and simultaneously lowering the risk of bleeding side effects when compared to other mechanisms *e.g.* inhibition of Xa and thrombin. Polymer-assisted solution phase synthesis of an α-ketothiazole library of the general form D-Phe–L-amino acid–L-Arg–α-ketothiazole was described.[646] These compounds were found to be potent, reversible-covalent inhibitors of tissue



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factor VIIa. The same research group described the polymer assisted solution phase synthesis of a library of aryl-substituted pyrazinones as potent and highly selective factor VIIa inhibitors.[647,648] Synthesis and crystal structures of substituted benzenes and benzoquinones[649] as well as 2-pyridones[650] as tissue factor VIIa inhibitors are described.

5.18 Topoisomerase I and II inhibitors

Topoisomerase II is an enzyme that catalyses changes in the topology of DNA *via* a mechanism involving the transient double-strand breaking and rejoining of phosphodiester bonds in DNA.

The mechanism associated with topoisomerase I involves the formation of single strand DNA break. Drug candidates have the ability to stabilize the topoisomerase–DNA cleavable complex. This drug-induced stabilization of the enzyme–DNA complex effectively converts these enzymes into cellular poisons. Thus, substituted dibenzo[*c*,*h*]cinnolines proved to be topoisomerase-I targeting anticancer agents.[651] The relaxation of positive superhelical tension of DNA structures by topoisomerases proved to be the key event required for RNA synthesis from chromatin template.[652] A series of new analogues of 3,9-acridinylamino)-5-hydroxymethylaniline (AHMA) were synthesized as potential antitumor agents inhibiting topoisomerase enzymes.[653] Tacrine, a widely used drug for treating Alzheimer's disease, inhibits topoisomerases and thus DNA synthesis to cause mitochondrial DNA depletion and apoptosis in mouse liver.[654] A series of 1,8-diazaanthraquinone derivatives bearing 3-dialkylaminomethyl or 3-(*N*-alkyl- or *N*-aryl) carbamoyloxymethyl substituents was synthesized. Their *in vitro* cytotoxic activities were evaluated against 8 human cancer cell lines. These compounds inhibited topoisomerase II mediated DNA relaxation, suggesting that this inhibitory effect be attributable for their cytotoxicity.[655] Inhibition of topoisomerase II with vepesid, an enzyme inhibitor, induced structural and functional reorganization of chromatin in miotically dividing spermatocytes causing desstructive effect on mouse spermatogenesis.[656] Hybrid compounds of psorospermin/quinobenzoxazine (A-62176) were synthesized and evaluated as DNA alkylating agents. The hybrid compounds showed, indeed, enhanced DNA alkylating activity.[657] Design, synthesis and biological evaluation of a series of flouroquinoanthroxazines with contrasting dual mechanisms of action against topoisomerase II and G-quadruplexes were described.[658]

Compounds interfering with the catalytic activity of the enzyme topoisomerase II (*e.g.* merborane) are expected to be active against both fast and slow growing cancers. Three series of 5-substituted 1,3-diphenyl-6-(ω-dialkyl- and ω-cycloaminoalkyl)thio-2-thiobarbiturates were synthesized as polysubstituted thioanalogues of merborane.[659] These analogues possess antiproliferative activity. Bis-naphthalimides represent a promising group of DNA-targeted anticancer agent. Different dimeric molecules of chromophore-modified bisnaphthalimides (containing two tetracyclic DNA-interchelating chromophores) were synthesized.[660] The strong binding interaction between the drugs and DNA perturbs the relation of supercoiled DNA by topoisomerases, but the test compounds do not promote DNA cleavage by topoisomerases. A new way was found for the synthesis of 4-hydroxymethyl-and 4-methoxymethylfuro[2,3-*h*]quinolin-2(14)-ones, the novel compounds inhibited topoisomerase-II, leading to a moderate antiproliferative activity in mammalian cells.[661] The novel antitumor compound NC-190 strongly inhibited the growth of FM3A cells, it can suppress the expression of the gene for thymidine kinase and simultaneously it inhibits topoisomerase-II.[662]

5.19 Trypsin and other serine protease inhibitors

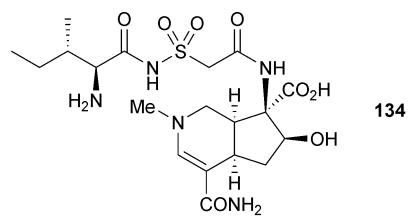
A novel major field of interest is the design of minimal units required for enzyme inhibition in structural biology and biotechnology. The successful design of a synthetic cyclic dodecapeptide corresponding to the Phe17–Val28 reactive site amino acid sequence of the low molar mass trypsin inhibitor micro-RTI-III from *Brassica napus* (rape) was performed. Micro-RTI-III is a new minimal trypsin inhibitor and may be regarded as a tool in protein structure–function studies for developing novel inhibitors.[663] 3-Formyl-4-hydroxyphenylguanidine-containing Schiff bases and their copper(II), zinc(II) and iron(III) chelates were synthesized. The novel compounds proved to be weak inhibitors of bovine trypsin.[664]

Proprotein convertases (PCs) are serine proteases with a subtilisin-like catalytic domain that are involved in the conversion of hormone precursors into their active forms. A cyclic peptide of 18 amino acids, derived from barley serine proteinase inhibitor (83 amino acids) was designed and evaluated as a new effective PC inhibitor.[665] The activity of α-chymotrypsin, a well-known serine protease, can be inhibited by a new class of inhibitors, hydroxyalkylpyrrols which contain an electron withdrawing group.[666] Low molecular weight cyanopeptides proved to be inhibitors of trypsin-like serine proteases.[667] Synthetic 4-amidinobenzylamine-based inhibitors with distinct selectivity for prototypical serine proteases showed anti-metastatic activity and it may be important for the future rational design of anti-proteolytic agents for cancer therapy.[668]

5.20 tRNA synthetase inhibitors

The emergence of bacterial strains with resistance to antibacterial agents (*e.g.* antibiotics) pressed the drug researchers to find novel agents with new modes of action. The family of bacterial amino acid t-RNA synthetases forms a novel target. A potent and selective series of substituted pyrazoles was discovered having high inhibitory potency on bacterial methionyl–tRNA synthetase and selectivity over human Met–tRNA synthetase.[669]

Twenty two analogues of the new anti-infective agent SB-203207 (**134**) have been prepared by total synthesis and evaluated as inhibitors of a range of tRNA synthetases.[670] The discovery of a new class of bacterial methionyl tRNA synthetase was recently reported (**135**). An early chemistry lead (**136**) with improved MRS inhibition and target-related antibacterial activity has been derived from it. Optimization of the aryl moiety in compound (**135**) led to the identification of a series of potent nanomolar inhibitors.[671]



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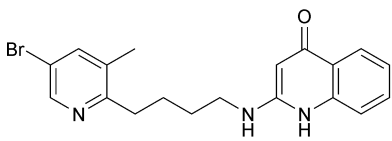

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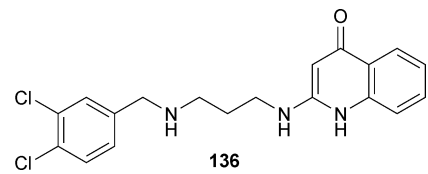

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5.21 Miscellaneous

Human chymose is a chymotrypsin-like serine protease showing exoproteolytic activity and producing a series of biologically active factors (*e.g.* angiotensin II,

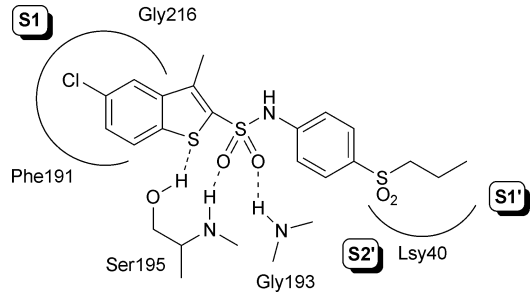

Fig. 26

inflammatory cytokines from precursors). A novel class of chymase inhibitor was identified through a substituent analysis of MWP00965, a weak chymase inhibitor.[672] The structure and binding mode of MWP00965 are shown on Fig. 26.

After structure optimization, the best benzo[b]thiophene-2-sulfonamide derivatives showed high potency ($IC_{50} \sim 56$ nM) and excellent selectivity for chymase compared to chymotrypsin and cathepsin-G. A review focused on the problem of the use of registered, orally active chymase inhibitors (NK301, BCEAB) for the treatment of cardiovascular disorders.[673] These inhibitors may represent an important development for the treatment of cardiovascular injury associated with mast cell degranulation. The possible role of mast cell chymase in organ fibrosis was examined using a bleomycin-induced pulmonary fibrosis model in mice.[674] Experimental results suggest that mast cell chymase may participate in the pathogenesis of pulmonary fibrosis, thus chymase inhibitors may be promising for treatment of pulmonary fibrosis in humans.

Mitogen-activated protein kinases play an essential role in oxidative burst-independent expression of pathogenesis-related genes in parsley.[675]

Recent studies have implicated a crucial role for tissue transglutaminase in the pathogenesis of Celiac Sprue, a disorder in the small intestine triggered by gluten in the diet. Design, synthesis and evaluation of gluten peptide analogues as selective inhibitors of human tissue transglutaminase were described.[676] The novel molecules contain acivicin or, alternatively, 6-diazo-5-oxonorleucine as warheads. The residue 6-diazo-5-oxonorleucine (DON) transforms as immunodominant gluten peptide into a potent inhibitor of tissue transglutaminase.[677] Kinetic analysis of the action of tissue transglutaminase on peptide and protein substrates revealed the complexity of the reaction (Gln hydrolysis, intramolecular transpeptidation, intermolecular transpeptidation, and transamidation by added nucleophile).[678] Coagulation factor XIII is also a transglutaminase catalyzing the crosslinking of fibrin chains and other processes. The pathogenic role of factor XIII is reviewed: the factor plays bivalent role in atherothrombosis.[679] An increased activity of factor XIII would favor the persistence of fibrin depositions and increased plaque burden, while on the other side it would reduce plaque vulnerability and the risk of downstream embolization.

Comparative molecular field analysis (CoMFA) and comparative molecular similarity analysis (CoMSiA) were used for understanding the antitumor activity of novel hydroxyl semicarbazide derivatives as ribonucleotide reductase (RNR) inhibitors.[680] This crucial enzyme catalyzes the reduction of ribonucleotides to deoxyribonucleotides. Design, synthesis and evaluation of novel compounds (phosphonoacetic acid esters and amide bioisosters of ribofuranosyl nucleoside diphosphates) were performed; these compounds are weak RNR inhibitors with low cytostatic and antiviral activity.[681] Ribonucleosides and xylonucleosides bearing a disulfide function on the sugar ring were synthesized.[682] Ribonucleosides belonging to the cytidine series were found to efficiently reduce deoxynucleotide triphosphate pools and serve as inhibitors of RNR. In addition, deoxyribonucleoside 2′- or 3′-mixed disulfides serve as prodrugs to target RNR and/or inhibit HIV reverse transcription.[683] Trimidox and didox, two recently synthesized specific inhibitors

of RNR synergistically enhanced the effect of temozolomide, an oral alkylating agent with good penetration of the BBB in malignant brain tumor cells.[684]

6. Phage library leads

Hydrolysis of β-lactam antibiotics by β-lactamase enzymes is the most common mechanism of bacterial resistance to antibiotics. A broad spectrum of peptide inhibitor of β-lactamase was identified using phage display and peptide arrays.[685] After two rounds of optimization, a linear hexapeptide RRGKYY was obtained possessing a K_i of 136 μM for inhibition PM-1 β-lactamase. The dramatic improvement in the NMR spectra of insulin-like growth factor I (IGF-I) in the presence of a peptide identified from a phage display library allowed the determination of a high-resolution solution structure for IFF-I molecules for the first time.[686] Phage-displayed peptides with high affinity for protein elongation factor Tu (EF-Tu) were selected from a library of $\sim 4.7 \times 10^{11}$ different peptides. Their binding affinity was optimized and detailed structure–activity relationships for multiple side chains of a polypeptide ligand were provided.[687] Novel inhibitors of *Pseudomonas aeruginosa* MurC enzyme necessary for proteoglycan monomer biosynthesis were identified from phage-displayed peptide libraries.[688]

Potent Grb2-SH2 domain antagonists were developed and further modified by constraining the backbone conformation and optimizing amino acid side chains of a phage library-derived peptide.[689]

7. Protein–protein interaction inhibitors: SH2 and SH3 domain ligands

Global optimization of conformational constraint on non-phophorylated cyclic peptide antagonists of the Grb2-SH2 domain was performed.[690] A new conformationally constrained L-tyrosine analogue as a potential scaffold for SH2 domain ligands was synthesized.[691] A β-amino-phosphotyrosyl mimetic was utilized in the design and synthesis of macrocyclic Grb2-SH2 domain-binding peptides[692] *via* macrolactamization of a highly functionalized, naphthyl-containing γ-amino acid analogue. A new group of potent Grb2-SH2 domain antagonist was identified that do not rely on phosphotyrosine or its mimics. These inhibitors may be used for the regulation of malignant cell growth by modulating Grb2-related Ras-signaling.[693] The construction of rigid spacers composed of amino propynyl benzoic acid building blocks was described. These spacers were used to link two phosphopeptide ligand sites binding the Syk SH2 domains with equally high affinity as the natural ligand.[694] Macrocyclization in the design of Grb2 SH2 domain binding ligands resulted in potent inhibitors in whole-cell system (Fig. 27).[695]

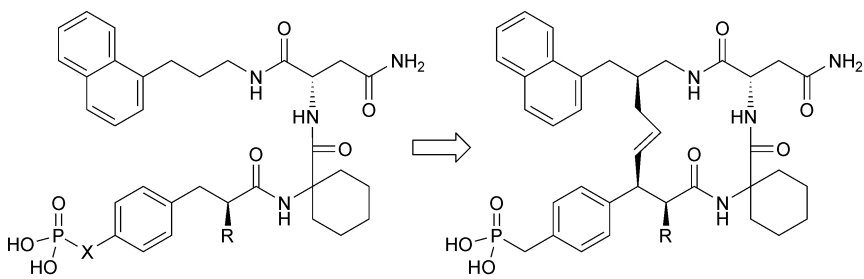

Fig. 27

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Cyclic, modified and conjugated peptides

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1 Introduction

The subject matter for this Chapter has again been allocated sub-headings that reflect main subject areas under which a reasonable number of publications have been cited. In the absence of a detailed keyword index, the sub-headings also provide continuity from previous volumes, so that searching the literature on certain topics might be made easier for the reader. This Chapter represents a change of coverage from previous volumes in that it covers the main papers over the two years 2003–04, rather than aim for a comprehensive coverage of one year's productivity as in the past.

Collecting the core references has again benefited from scanning CA Selects on Amino Acids, Peptides and Proteins (up to issue 16, 2004),[1] while the development of computer scanning of the Web of Knowledge data bases[2] has contributed greatly to the aim of securing a more comprehensive coverage. Proceedings from various symposia (such as the 18th American Peptide Symposium[3] and the 28th European Symposium in Prague) have not been rigorously reviewed until material arrives in refereed Journals.

All aspects of the subject matter of this Chapter benefit greatly from the developments associated with solid phase synthesis of peptides (SPPS). The current status of SPPS in the synthesis of the full range of peptides has been reviewed[4a] and includes reference to special techniques developed for cyclic peptides. A more recent review[4b] has also updated the current trends in peptide coupling reagents. The ethos of peptidomimetic work through the introduction of conformational constraints is well exemplified by chosen examples in an authoritative review.[5] A review[6] of the role of natural products in drug discovery contains many examples covered by the subject matter of this Chapter.

2 Cyclic peptides

2.1 General considerations

The macrocyclisation of linear precursors forms a critical step in the synthesis of cyclic peptides and depsipeptides. The most successful and efficient methodologies developed for this task have been the subject of review,[7] while the combination of SPPS and microwave-assisted synthesis for this purpose has been assessed.[8] In the sulfhydryl displacement of an aromatic fluorine, microwave assistance increased the yield form 50% to an average of 75%. A more focused study of total synthesis of selected naturally occurring peptides has also appeared.[9] With the aim of constructing bicyclic homodetic libraries, based on the tachykinin NK2 antagonist MEN 10627 [cyclo(Met–Asp–Trp–Phe–Dpr–Leu)cyclo(2β-5β)], an extra level of orthogonal protection schemes was desirable.[10] Scheme 1 summarises a typical strategy based on SPPS [two cycles based on Fmoc/Bu^t and side chain protection with Aloc / All and Dmb (2,4-Dimethoxybenzyl)]. The desired products were only obtained when the small ring was first formed followed by the larger ring, and in the absence of any *N*-methylated or D-residues. However *N*-alkylation had a stabilising influence on the cyclic peptidomimetics produced[11] by the SET-promoted photocyclisation of N-terminal phthalimides as summarised in Scheme 2. Libraries of heterodetic cyclic peptides have been constructed[12] through intramolecular oxime formation as represented by (**1**).

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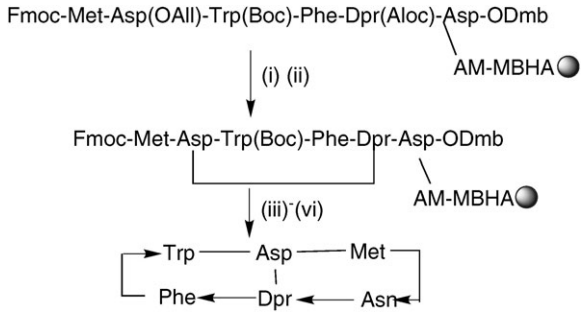
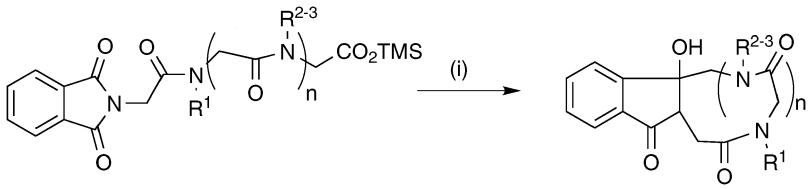
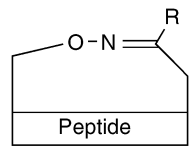


Scheme 1 Reagents (i) $Pd(PPh_3)$ (ii) PyBOP/HOBt/DIEA (iii) piperidine/DMF (iv) TFA/CH_2Cl_2 (v) PyBOP/HOBt/DIEA (vi) TFA/H_2O-i-Pr_2SiH-phenol.

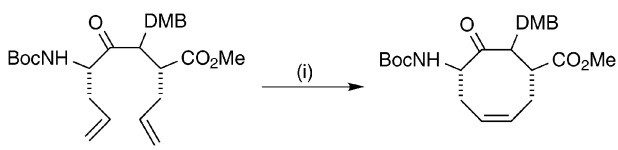


Scheme 2 Reagents (i) hv, nBu_4NOH, 35% H_2O-MeOH.



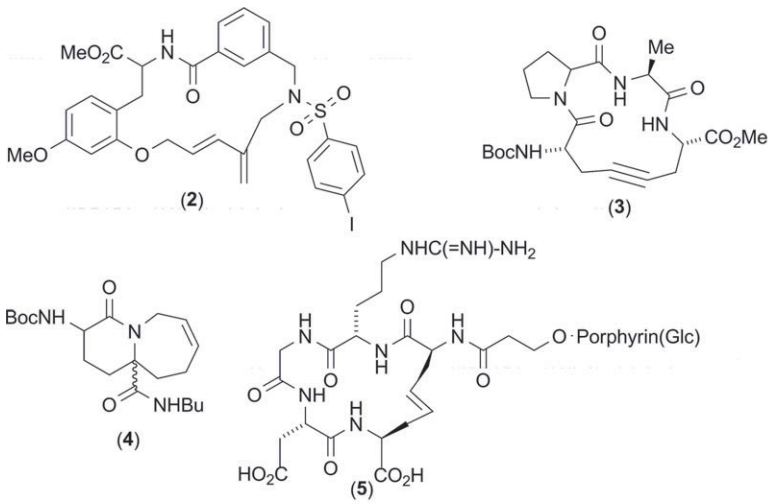
(**1**)

The application of ring-closing metathesis (RCM), based on Grubbs' ruthenium catalysts has gathered further examples for restricting the conformation of peptides by macrocyclisation. In a study[13] to design and synthesise β-turn mimetics, one of the series revealed the relatively rare Type VIa β-turn. The key step used is summarised in Scheme 3, with the best yields being secured by the original benzylidene ruthenium catalyst rather than the second-generation imidazoline catalyst. Novel macrocyclic peptidomimetics with 15–18 membered rings, such as (**2**) have been produced[14] by RCM, with more stereospecificity in ene formation being obtained using the second generation catalyst. Rapid access[15] to Fragment A of the cryptophycins with high *E*-selectivity has been achieved using RCM on bishomoallylic alcohols. An alkyne-forming version of RCM has produced[16] geometrically pure cystine isosteres such as (**3**), which on conformational analysis appear to be more rigid than their sulfur counterparts. A combination of a sequential Ugi reactions and RCM has yielded[17] bicyclic lactams such as (**4**), while the cyclic RGD ring in a glucosylated porphyrin (**5**) designed for cancer



Scheme 3 Reagents (i) $Cl_2Ru(PCy_3)_2$ = CHPh, DCM, reflux, 1 day.

phototherapy, has been formed *via* an RCM step.[18] A new class of ureapeptoid-containing macrocycles has been inaugurated *via* the RCM approach.[19]

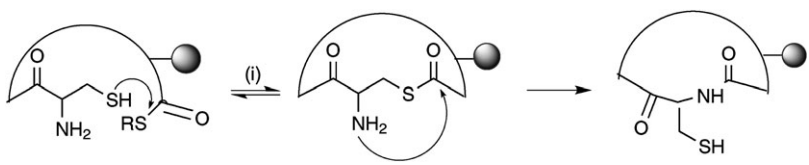


$HSCH_2CO$-Tyr-D-Ala-(Lys-D-Ala)$_4$-Gln-$NHCH_2CH_2SH$

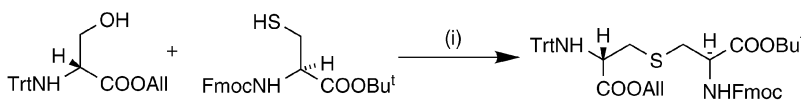
(**6**)

To quench the demand for open/closed states of cyclic peptides, thiol groups have been added[20] to both termini of an alternating D, L-residue peptide (**6**), so that they can cyclise, *via* a disulfide bond, to a structure with all amino acid side chains on the outside of the ring. The cyclic model peptide, cyclo (Cys–Thr–Abu–Gly–Gly–Ala–Arg–Pro–Asp–Phe) has been synthesised[21] using on-resin native chemical ligation as summarised in Scheme 4. Continuing interest in the lantibiotics, the thioether-linked antimicrobial peptides, has spurred on developments in the construction of the thioether bridge. Thus the lack of reactivity of sulfur nucleophiles towards a 'Mitsunobu-activated' serine has been overcome[22] by the use of catalytic zinc tartrate which enhances the nucleophilicity of the thiol. The key step is summarised in Scheme 5. A one-pot protocol to generate on solid phase, cyclic peptides with a thiosulfide bridge has been reported,[23] which included a microwave-assisted cyclisation stage. Scheme 6 summarises the thioether-forming step.

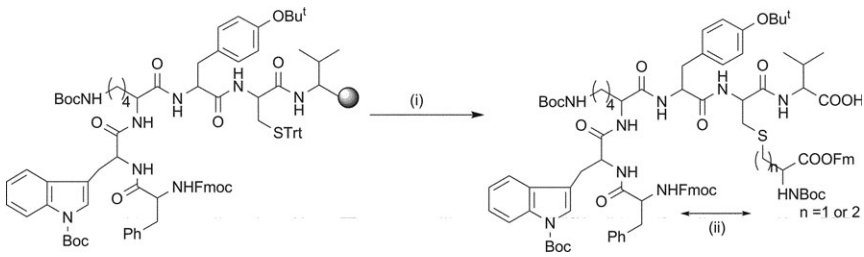
Peptides have also been constrained by cyclisation using a Mannich condensation reaction[24] between an N-terminal amino group and a tyrosyl side-chain as represented in Scheme 7. Stages up to structure (**7**) could be accomplished on solid



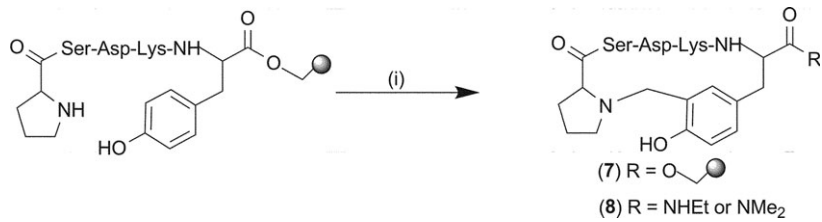
Scheme 4 (i) pH 7.5.



Scheme 5 Reagents: ADDP, Me_3P, Zn tartrate (0.2 eq) rt. 7 days (50%).

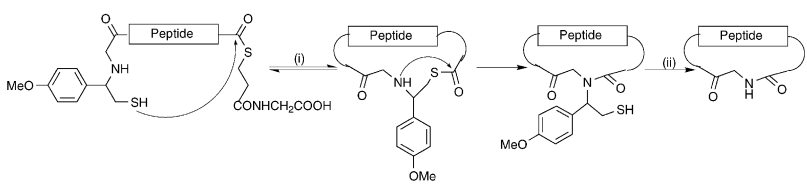


Scheme 6 Reagents: (i) (a) TFA/TES/DCM (b) BocNHCH[$(CH_2)_nCH_2$l]COOFm (ii) (a) piperidine (b) TBTU/DIEA.

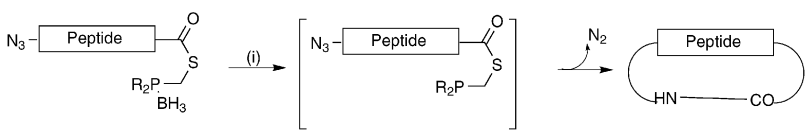


Scheme 7 Reagent: 37% HCHO, H_2O/THF, conc HCl (30/69/1).

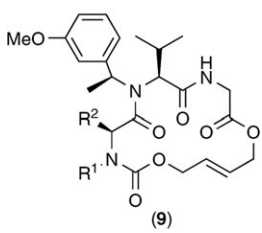
phase, with the final cyclic constructs being obtained from the resin, as in (**8**), using appropriate amines. The usual need for a terminal Cys residue to enable cyclisation *via* the native chemical ligation method, can be replaced[25] by a removable auxiliary thiol source as exemplified by Scheme 8 being the summary of the cyclisation of H–Gly–Ser–Pro–Tyr–Ser–Ser–Asp–Thr–Thr–Pro–Ala–OH. Expansion in the demands for construction of peptide libraries has also seen a corresponding popularity of the Ugi multi-component reaction. This approach[26] secured the linear precursor to cyclic peptide (**9**), which was cyclised at the ene bond *via* the RCM approach using Grubbs' catalyst. An on-resin Ugi four-component reaction[27] followed by an intramolecular nucleophilic aromatic substitution has secured access to biaryl-ether containing macrocycles such as (**10**). The synthesis of cycloisodityrosines such as (**11**), constituents of many anti-tumour cyclic peptides has been brought about[28] *via* a copper(II) acetate/DMAP mediated diaryl ether formation from precursor phenylboronic acids. Both medium-sized lactams and lactams derived from β-alanine-containing peptides have been formed[29] using the intramolecular Staudinger ligation summarised in Scheme 9.

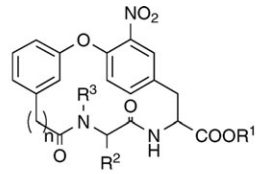


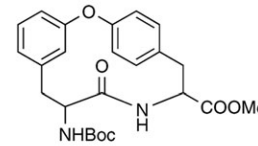
Scheme 8 (i) pH 7.4 (ii) HF.



Scheme 9 Reagent: (i) DABCO.

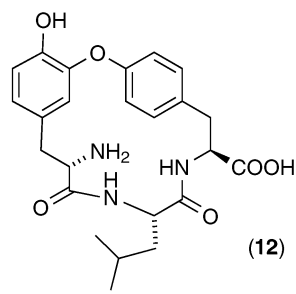






The application of mass spectrometric techniques to the sequencing of cyclic peptides often requires some initial modification due to the potential for multiple ring openings. In a study[30] of the preferred binding sites of biotin esters to polymyxin antibiotics, the biotin derivatised cyclic peptides aided the identification of fragments in an electrospray/tandem MS/MS analysis. In a collisionally activated dissociation (CAD) study[31] using an ion trap, protonated and metal-complexed cyclic peptides have been analysed. For cyclosporin A, nickel and lithium complexes gave additional sequence information, while for depsipeptides, sodium and lead complexes were superior to the protonated peptide. Silver, nickel and strontium gave enhanced abundances of key fragment ions for cyclic lipopeptides. Automated computer analysis[32] of mass spectra has assisted in achieving a rapid identification of cyclic peptide library members in a sonic spray ion trap MS and an MS/MS analysis of a single compound on a bead. A microwave-assisted modification of the Akabori hydrazinolysis, has been shown[33] to cleave cyclic oligopeptides to give linear analogues which can be sequenced by ESI-MS/MS or FAB-MS/MS. Liquid chromatography-ESI-MS has enabled the quantification[34] of saxitoxin, anatoxin-A, domoic acid, nodularin, microcystins, okadoic acid and dinophysistoxin-1 in a single chromatographic run.

A stochastic search algorithm has been derived[35] for exploring the multidimensional space of cyclic peptides, while an algorithm named SCSA[36] has analysed the conformational space *in vacuo*, of the marine cyclotripeptide ranieramide (**12**) from Vanuatu sponge. NMR methods in combination with molecular dynamics simulations[37] have been used to define the solution conformation of contryphan –Vn, a Ca^{2+} dependent K^+ channel modulator, containing two cysteine S–S bridges.

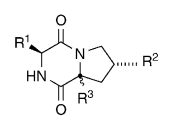


Topics of general interest reviewed during this period, have been the cyclopeptide contents of the seeds of *Vaccaria segetalis*,[38] the peptide lactones, cyclic peptides and depsipeptides isolated from marine sponges,[39] and the inhibition of serine proteases by cyclic peptides from bacteria.[40]

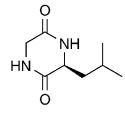
2.2 Cyclic dipeptides (dioxo- or diketo-piperazines)

Studies in the marine environment are actively revealing interesting structures in the cyclic dipeptide context. Exploitation of Chinese sponges has been encouraged,[41] and three families of diketopiperazines with structures (**13–15**) have been found[42] in the marine sponge *Ircinia variabilis*. A dioxopiperazine alkaloid golmaenone (**16**) has

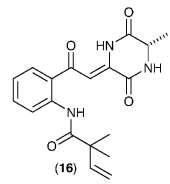
been isolated[43] from the marine-derived fungus *Aspergillus* sp, while cultures of larvae from molluscs have yielded[44] five cyclic D, D-dipeptides with strong antibiotic activity against *Vibrio anguillarum*. Four were based on cyclo (D-Pro-D-X), with X=D-Phe, D-Leu, D-Ile and D-Val, with the fifth having the structure, cyclo (*trans*-D-4OHPro-D-Phe). A novel anti-fungal diketopiperazine (**17**) has been identified[45] in a marine fungus M-3 from *Porphyra yezoensis*, while the brominated cyclo (6-BrTrp-Arg) and cyclo (6-Br-8-enTrp-Arg) from the marine sponge *Geodia barretti* have turned out[46] to have good anti-fouling activity.



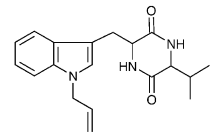
(**13**) R^3 = ⁃H (**14**) R^3 = ···H



(**15**)

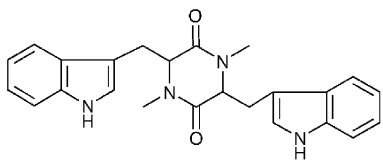


(**16**)

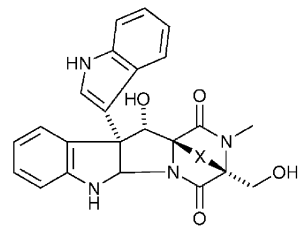


(**17**)

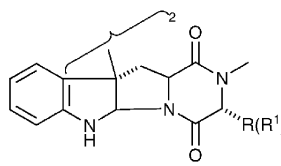
Dehydro-cyclic dipeptides have been subject of a review[47] in Japanese, and two new cyclopeptides, arenariphilins A, cyclo (Thr–Gly), and B which is a cyclohexapeptide, cyclo (Ser–Gly–Ser–Ile–Phe–Phe), have been isolated[48] from the whole plants of *Arenaria orephila*. Diketopiperazine Sch 725418 (**18**) has been found[49] in *Micromonospora* sp., and novel cytotoxic thioketopiperazines, T988 A, B and C (**19–21**) have been identified[50] in *Tilachlidium* sp. The dimeric dioxopiperazine (**22**) has been isolated[51] from *Aspergillus niger*.



(**18**)

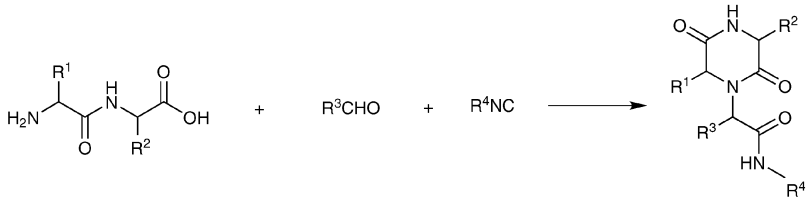


(**19**) X = S_3 (**20**) X = SMe, SMe (non cyclic)
(**21**) X = S_2



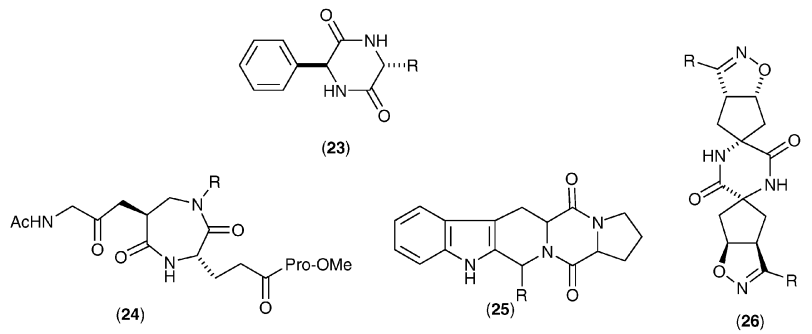
(**22**) R = Me_2CH R^1(in other half) = Me_2CHCH_2

The diverse applications of diketopiperazines have been reviewed,[52] and an example for the use of the Ugi 4-centre 3-component reaction in their synthesis has been described.[53] Scheme 10 summarises the key reaction. The key step in forming D-phenylglycyl dioxopiperazines (**23**) was the penicillin acylase-catalysed synthesis[54] in aqueous medium of the precursor linear dipeptides. A 7-membered 1,4-diazepine-2, 5-dione has been synthesised[55] using a BAL linker on an amino-methyl polystyrene resin loaded with both an α- and β-amino acids. Cyclisation was carried out on the solid support using sodium methoxide/methanol. A solution phase equivalent was also carried out using EDC/HOBt for cyclisation. The same scaffold, this time as a 3,6-disubstituted-1,4-diazepan-2,5-dione core as in (**24**) has been constructed[56] *via* a Ser-containing dipeptide linked to a hydroxylamine resin, followed by cyclisation of the hydroxylhydroxamate under Mitsunobu conditions, and microwave heating. The latter form of heating also gave higher yields[57] in a

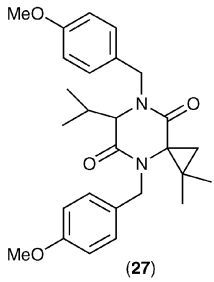


Scheme 10 (i) −40 °C, R.T., CF_3CH_2OH

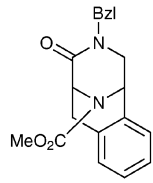
dimerisation strategy using 2,5-diketo piperazines as scaffold, and in synthesis[58] of the tetrahydro carboline DKP (**25**). Diketopiperazine (**26**) was the result[59] of a couple of diastereoselective 1,3-dipolar cycloaddition steps which control alkene face-selectivity. Proline anchored to polyethylene glycol monomethyl ether provided[60] the first step to dipeptide formation with a series of Fmoc amino acids in the presence of DCC, which after deprotection yielded high yields of piperazine diones. Standard cyclisation methodology has also furnished[61] cyclo (Pro–Leu), cyclo (Pro–Ile) and cyclo (Trp–Pro).



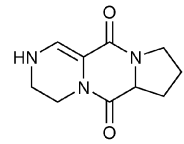
N-Methylation of diketopiperazines[62] has been shown to improve solubility, presumably by reducing aggregation due to H-bonding. Several successful syntheses of the *N*-methylated analogues were reported using on-resin techniques and piperidine/DMF for cyclative release from the resin. A pseudo diketopiperazine cyclo (PheΨ[CH_2NH]Leu) was also synthesised. Diketopiperazinecyclopropane (**27**) has been prepared[63] *via* the conjugate addition of a phosphorus ylide to (6*S*)-*N,N′*-bis(*p*-methoxybenzyl)-3-methylene piperazine-2,5-dione and used for asymmetric synthesis of 1-aminocyclopropane-1-carboxylic acids. Improvements[64] have been made to increase the efficiency of the aldol condensation between 1,4-diacetylpiperazin-2,5-dione and aromatic aldehydes. Conjugated additions[65] of lithiated bislactim ethers of cyclo (Gly–Val) and cyclo (Ala–Val) to α-, β-, or α,β-substituted vinylphosphonates have given stereoselective access to a series of mono- and di-substituted 2-amino-4-phosphonobutanoic acids, while synthesis of the bridged piperazine-3-one (**28**) was the result[66] of selective reduction of one keto group in the dioxopiperazine ring of 2,5-disubstituted piperazine-3, 6-dione. The unsaturated diketopiperazine (**29**) has been the focus[67] of diastereoselective hydrogenation studies with various noble metals supported on charcoal. Hydrogenation over Pd, Rh and Ru catalysts proved to be the most efficient, all giving a preponderance of the (*S*) configuration at both chiral atoms. Tyrosine hydroxylase converted[68] cyclo (Tyr–Tyr) to its DOPA equivalent by aromatic ring hydroxylation, while multifunctional pyroglutamides can be synthesised[69] by opening up the diketopiperazine ring of (**30**) with primary diamines. In routine peptide synthesis, diketopiperazine formation is usually an unwanted pathway, but on using 2-(trimethylsilyl)isopropyl esters for C-terminal protection, diketopiperazine formation is greatly reduced.[70] A comparison[71]



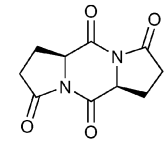
(**27**)



(**28**)



(**29**)



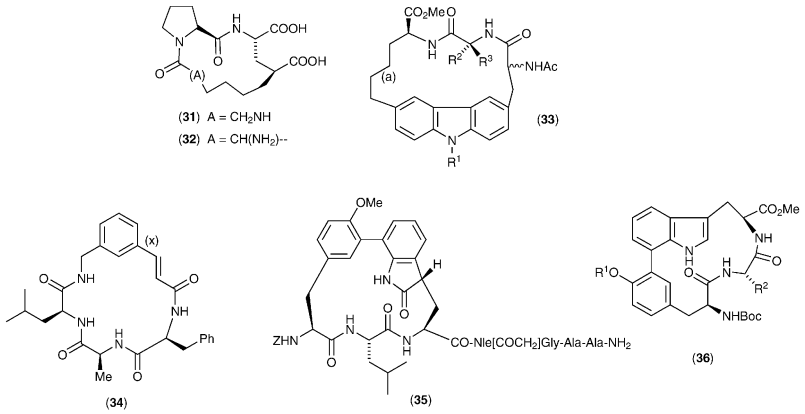
(**30**)

between the geometry of 2,5-dioxopiperazine-1,4-diacetic acid derived from *ab initio* HF-LCAO quantum-mechanical calculations and its X-ray crystal structure, showed the 6-membered ring to be more puckered in the theoretical study. Calculations at the B3LYP/6-311 + G(d,p) level of theory, on the reactions between MeS radicals and the diketopiperazines of Gly, Ala and Sar (H-atom abstraction by MeS radicals) have shown[72] the cyclo(Ala–Ala) to be the most reactive. Ultraviolet resonance Raman spectra[73] showed perturbed amide IIc frequencies, reflecting the kinematic mixing of the amide coordinates into in- and out-of-phase modes for cyclo (Gly–Gly) and cyclo (Gly–Pro). Equilibrium constants between glycylglycine and its diketopiperazine have been determined[74] using a FT-IR flow reactor at 310–330 °C and 275 bar.

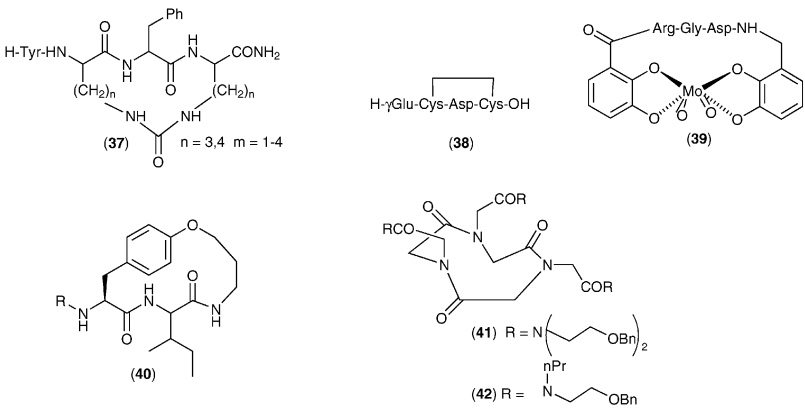
2.3 Cyclotripeptides

Homodetic cyclic peptides with only three residues are so conformationally-strained that only a few examples have been synthesised. One of these is cyclo tri-L-proline, which has the ability to form *cis*-bonds. Theoretical comparisons with crystal data have been explored[75] and the synthesis and conformational analysis of cyclo (4*S*-aminoproline)$_3$ have been reported,[76] which is an example of a bowl-shaped scaffold with three amino groups in the side chain available for further development. Best yield obtained in the cyclisation of its linear precursor was 71% using HATU/Pr^i_2NEt for 2 hr. Cyclo (Gly–Pro–Glu) and a cyclic tetrapeptide, cyclo (Gly–Ser–Pro–Glu), both having moderate antibacterial activity, have been characterised[77] in cell extracts of a *Ruegeria* strain of bacteria associated with the sponge *Suberites domuncula*. Nature[78] has put together a very interesting cyclotripeptide structure in the form of psychrophilin A [cyclo (NO_2Trp–Pro–*o*-aminobenzoyl)] isolated from the fungus *Penicillium ribeum* which also biosynthesises the cyclopentapeptide, cycloaspeptide D [cyclo (Ala–MePhe–Val–MeTyr-*o*-aminobenzoyl)].

There is less strain apparent in rings enlarged[79] *via* RCM using Grubbs catalyst followed by hydrogenation to give (**31**) and (**32**), as it has been revealed that the *cis-trans* Pro amide rotamer ratio in (**31**) was 35:65 while in (**32**) it was all-*trans*. An RCM reaction at position (a) in (**33**) was also instrumental[80] in giving access to this new class of cyclic peptoids. Only moderate to poor antibacterial activities were shown by these compounds. A Heck reaction[81] between a 3-bromobenzyl group at the C-terminus and an acryloyl group at the N-terminus has yielded two cyclic peptides based on macrocyclisation at point (x) in (**34**). Side chain cyclisation[82] at the biphenyl link in (**35**) using a Suzuki cross coupling reaction, has yielded a TMC-95A ketomethylene analogue which had a lower inhibitory value than the parent molecule. Three constrained analogues of TMC-95A with general formula (**36**) were constructed[83] *via* macrocyclisation at the biaryl link using Ni(0)-mediated coupling. A preliminary communication[84] summarises the solid phase synthesis of cyclo (βAsp-β^3hVal-β^3Lys) initiated by attachment of the α-COOH of βAsp to the resin and cyclisation between the other Asp COOH and β^3 Lys using HBTU/HOBt.



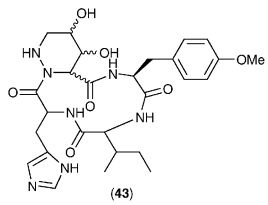
In a series of side-chain to side-chain cyclised dermorphin analogues (**37**), the D-Lys[2], Dab[4] ($n = 4$, $m = 2$) analogue was 210 times more active than enkephalin in the guinea-pig ileum assay.[85] Constraining a glutathione analogue in a 11-membered ring as in (**38**) did not result in improved binding to glutathione reductase.[86] Attachment of catechol ligands at both termini of tripeptides allows macrocyclic molybdenum (VI) complexes to be constructed[87] which can constrain the conformation of well known naturally-occurring triads such as RGD (**39**) or the WKY sequence of urotensin II. Constrained templates of tripeptides produced as a library based on structures such as (**40**), have been designed[88] to preorganise the ligand structure, thereby organising the local enzyme environment in inhibition studies. This approach should minimise the problems of induced fit and reduce co-operative effects caused by changes in adjoining interactions. *N,N′,N″*-Trisubstituted cyclotriglycines[89] have their substituents on nitrogen inclined in the same direction so analogues such as (**41–42**) show high affinity with Ca^{2+}. The precursor synthons to this series of compounds were the *N,N′N″*-triallyl analogues.



2.4 Cyclotetrapeptides

Nature has provided some interesting structures in this category, as exemplified by: the antifungal glomecidin (**43**) from *Streptomyces* sp. H698 Sy2;[90] cyclo (Ile–Pro–Leu–Pro) from an actinomycete[91] of the marine-derived genus, *Nocardiopsis*; the cyclotetrapeptide (**44**) from a bacterium associated with the sponge *Ircinia muscarum*.[92] The applications of the cyclotetrapeptides, HC-toxin, Cyl1/2, WF-3161, trapoxin and chlamydocin, and their analogues, as inhibitors of histone deacetylase

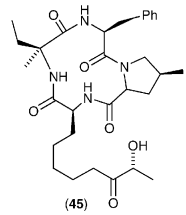
(HDAC) have been reviewed.[93] Others have plotted[94] the development of HDAC inhibitors for anti-cancer chemotherapy and differentiation therapy, while nature has produced[95] cyclotetrapeptide FR235222 (**45**) from the fungus *Acremonium* sp. No. 27082, which is a HDAC inhibitor with immunosuppressive effects. Celogentin K (**46**), isolated from the seeds of *Celosia argentea*, has a cyclotetrapeptide as its core,[96] and in the same paper the related bicyclic peptide moroidin's structure has been confirmed by X-ray analysis. New inhibitors of HDAC containing sulfhydryl groups, based on (**47**), and related to CHAP31 and FK228, have been shown to exhibit[97] potent activity. Incorporating trifluoromethyl and pentafluoroethyl groups instead of the sulfur groups in (**47**) also produced[98] potent HDAC inhibitors. The introduction of side-chain to tail constraints[99] increased the potency of compounds such as (**48**) as compared to the linear analogue in inhibition of cyclin A and cyclin E-associated cyclin-dependent kinase 2 activities.



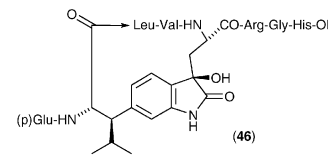
(**43**)

cyclo [Pro-Pro-Tyr-Tyr]

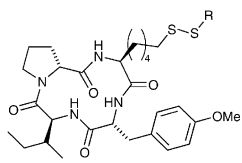
(**44**)



(**45**)



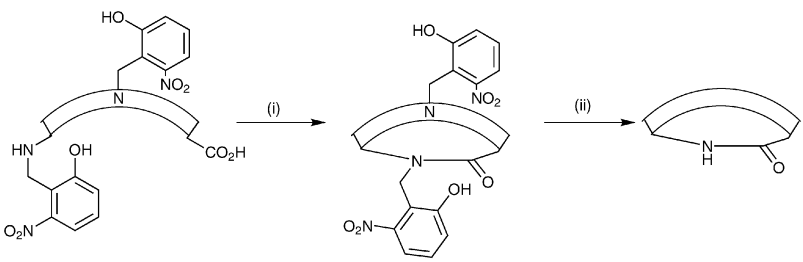
(**46**)



(**47**)

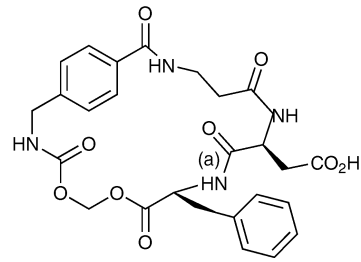
(**48**)

Always useful in this field, are assessments of reaction conditions that maximise the yield at the macrocyclisation stage. So it is useful to note that cyclodimerisation products were eliminated in an on-resin approach[100] to making a series of RGD-containing cyclotetrapeptides of the general formula cyclo (Xaa–Arg–Gly–Asp), where Xaa = Ala, Phe, Phg, D-Ala, D-Phe and D-Phg. The strategy involved anchoring the Asp side chain to the resin (Wang resin preferred), and using the Fmoc/Bu^t/OAl protection scheme, it was shown that activation by TBTU/DIPEA gave the best results. A new strategy[101] as summarised in Scheme 11 has enabled the difficult formation of cyclo (Tyr–Arg–Phe–Ala) to be accomplished, while the bridged dicatechol ligand approach seen in (**39**) has been extrapolated[102] to a segetalin-based sequence, Trp–Ala–Gly–Val. A cyclic prodrug (**49**) carrying the



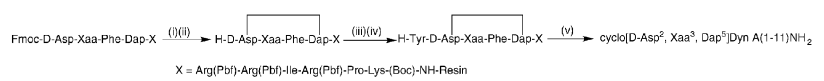
Scheme 11 (i) Cyclisation BOP/DIEA (ii) hν/1% AcOH.

RGD sequence has been synthesised[103] by cyclisation (26% yield) of a linear precursor at position (a) in (**49**) using HBTU/DIEA/DMF.

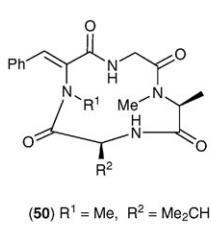


(**49**)

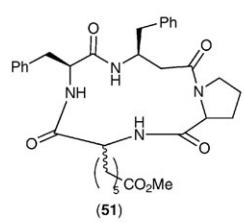
Tentoxin (**50**) and analogues have been synthesised,[104] where the two key steps of dehydration to form the didehydro residue and *N*-alkylation, took place whilst the sequence H–MeAla–Leu–(βOH)Phe–Gly–O–Resin was attached to a Wang resin. Final cyclisation off-resin was carried out using DIPCDI/HOBt/DIEA. The conformational strain in the 12-membered cyclotetrapeptide ring still hampers universal acceptance as a β-turn motif. Appropriate enlargement into a 13-membered cyclic motif can be made using β-amino acid units as exemplified[105] by scaffolds such as (**51**) (using BOP for cyclisation), which have side-chains orientated to mimic a number of natural products. The right hand side cyclic segment of moroidin has been synthesised[106] in fully protected form (**52**), the final cyclisation step being carried out between Arg and Trp using DPPA/Et_3N in 73% yield. The key steps to form the unusual Trp C-2/His N-1 link involved a displacement of 2-chloroindole with nucleophilic histidine, a Horner-Wadsworth-Emmons reaction followed by asymmetric hydrogenation. Side-chain cyclisation between positions 2 and 5 in a series of cyclic dynorphin analogues has been rationalised[107] as illustrated by Scheme 12, so that substitutions at position 3 could be monitored. Apart from the Pro^3 analogue, all of the cyclopeptides exhibited full agonist activity in the adenylyl cyclase assay using cloned κ-opioid receptors. Synthesis[108] of morphiceptin cyclic analogues (**53**) and (**54**) has allowed their electrophysiological activities to be monitored. They showed exclusive activity on μ-opioid receptors. Cyclen (1, 4, 7, 10-tetraazacyclotetradecane) derivatives have been used diagnostically and therapeutically as mimics of the binding site of metalloproteins, and can now be synthesised[109] (*e.g.* **55**) by on-resin reduction of the corresponding cyclotetrapeptide using BH_3.THF at 65 °C. Reduction of cyclo (X-β-Ala-X-β-la), with $LiAlH_4$/THF, where X = Gly, Ala, Leu, Val, or Phe has given a series[110] of C-substituted cyclams (1,4,8,11-tetraazacyclotetradecanes). An oxime resin linked to linear peptide precursors *via* a Gly residue figured[111] in the synthesis of RGD-furanoid sugar amino acid cyclic peptides, with cyclopeptide (**56**) showing the most promising activity in inhibition of the $\alpha_v\beta_3$ (IC_{50} 1.49 μM) and $\alpha_{IIb}\beta_3$ (IC_{50} 384 nM) integrins. RGD mimetics have also been 'tied back' as cyclic pro-drugs,[112] represented by (**57**), with (**57** $n = 7$) being the most stable, with improved transport and antithrombotic properties. Cyclocondensations[113] of dipeptide esters at Ni(II), Pd(II) and Cu(II) templates have given dianionic square-planar complexes of de-protonated cyclotetrapeptides, with structures being confirmed by the X-ray data on [(cyclo Gly-β-Ala-Gly-β-Ala-$4H^+$)Cu]$(PPN)_2$.



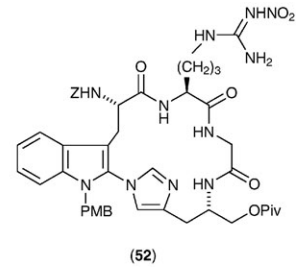
Scheme 12 Reagents (i) PyBOP/HOBt/DIEA, (ii) Piperidine, (iii) Fmoc-Tyr(OBu^t)-OH/PyBOP/HOBt, (iv) piperidine (v) TFA.



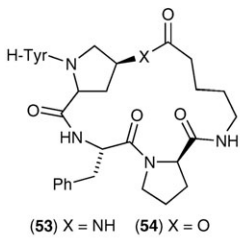
(**50**) R^1 = Me, R^2 = Me_2CH



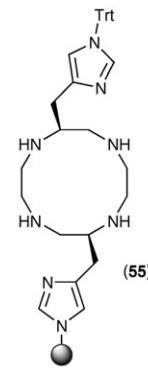
(**51**)



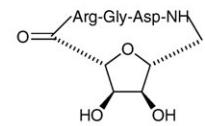
(**52**)



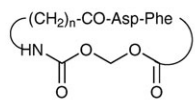
(**53**) X = NH (**54**) X = O



(**55**)

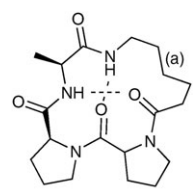


(**56**)



(**57**) n = 1, 3, 5 and 7

The self-assembly of cyclo [(-β^3-HGly)$_4$] has been investigated theoretically,[114] with the conclusion that the synergetic effect of adding monomers could facilitate the enhancement of favourable interactions of the nanotube. Selective inhibitors of estrogen receptor α, such as H–Lys–cyclo (D-Cys–Ile–Leu–Cys)–Arg–Leu–Leu–Gln–NH_2, through their disulfide bridge, allow a quasihelical conformer to be formed to enhance binding to the receptor,[115] while the disulfide bridge in Ac–Cys–Pro–Xaa–Cys–NH_2, X = Phe, His, Tyr, Gly and Thr and in Ac–Cys–Gly–Pro–Cys–NH_2 confers an unexpected rate enhancement for *cis*/*trans* isomerism across the Cys–Pro bond as determined by magnetisation transfer NMR spectroscopy.[116] Spectroscopic studies[117] on three cyclotetrapeptides, cyclo (Leu–D-Ala–Xaa–D-Ala), with X = Leu, Lys or Glu, confirmed that the alternating L–D sequences adopt open β-turn conformations. A molecular mechanics computational approach[118] has given good predictions of the conformational states of cyclo $(Gly)_4$, cyclo $(Ala)_4$, cyclo (Sar)4, cyclo $(Sar–Gly)_4$ and tentoxin, which agree well with experimental data. The tendency for tripeptides based on D-Pro–L-Pro to exist as 3_{10} helical structures has been retained[119] in the cyclotetrapeptide (**58**) which was synthesised by ring-closing metathesis at point (a) followed by hydrogenation.

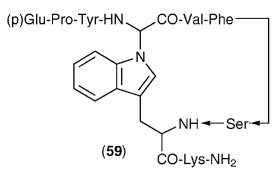


(**58**)

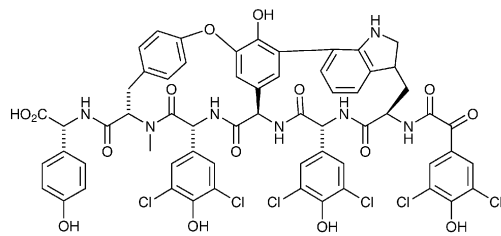
2.5 Cyclopentapeptides

The roots of *Pseudostellaria heterophylla* have yielded[120] a novel cyclopentapeptide, cyclo (Ala–Gly–Pro–Tyr–Leu), while new antitumour cyclic astin analogues has

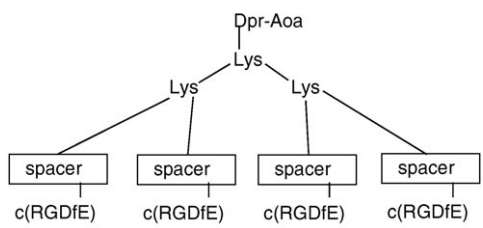
been synthesised.[121] The analogues prepared were, cyclo (Pro–Thr–Aib-β^{3}Phe–Abu), cyclo (Pro–Thr–Aib-β^{3}hPhe–Abu), cyclo (Pro–Thr–Aib-β^{3}hPheΨ[CH_2-SO_2NH]-Abu) and cyclo Pro–Abu–Ser-β^{3}hPhe Ψ[CH_2SO_2NH]–Abu). The latter analogue had comparable activity to astins A and B, probably due to the flexibility in its backbone allowing it to assume the bioactive conformation of astin B. Cytotoxic cyclic peptides from *Dianthus superbus* have been identified[122] as cyclo (Gly–Pro–Phe–Val–Phe) (dianthus F) and its higher analogues cyclo (Gly–Pro–Phe–Tyr–Val–Ile) and cyclo (Gly–Pro–Ile–Ser–Phe–Val) (dianthus C and E) and cyclo (Gly–Ser–Leu–Pro–Pro–Ile–Phe) (dianthus D). The seeds of *Celosia argentea* have yielded[123] celogenamide A (**59**). Segetalins B [cyclo (Gly–Val–Ala–Trp–Ala)] and G [cyclo (Gly–Val–Lys–Tyr–Ala)] have been synthesised,[124] with the most efficient ring closure taking place between Val and Gly in *ca*. 23% yield using DPPA in MeCN. The first stereoselective total synthesis of the anti-HIV agent chloropeptin 1 (**60**) has been reported.[125] Construction of the two macrocycles in (**60**) was achieved using a Cu-mediated biaryl ether formation and a Pd-mediated cross-coupling reaction.



(**59**)


(**60**)

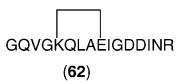
Cyclo (Pro–Tyr–Leu–Ala–Gly) and cyclo (Ala–Tyr–Leu–Ala–Gly) have been synthesised[126] by using a Ag^+ -assisted cyclisation of a phenacyl derivatised thioester at the Gly carboxyl of the linear precursor. Two complementary routes using either a Wadsworth-Emmons reaction or a Pd-catalysed coupling reaction have been found[127] for the synthesis of α,β-didehydrotyrosine within the southern segment of cyclotheonamide C. Increased stability to enzymic attack is a feature[128] of four cyclopentapeptides, cyclo (Ala–Arg–Pro–Ala–Lys), cyclo (Gln–Arg–Pro–Ala–Lys), cyclo (Gly–Arg–Pro–Ala–Lys) and cyclo (Lys–Arg–Pro–Ala–Lys), related to a fibrinogen fragment. A H-bond between the NH of Lys and the CO of Arg is suggested as the source of the stabilisation, while the cyclisation steps to the analogues had to rely on the older DCC/NMM coupling conditions. With the aim of designing RGD peptides which can bind polyvalently to several integrins, multimeric cyclic peptides such as (**61**) have been synthesised[129] by solid phase methodology and include an aminoxy group for versatile ligation of radiolabel or fluorescence labels. Direct radiofluorination of cyclo (Arg–Gly–Asp–D-Phe–MeVal) with ^{18}FAcOF has allowed[130] a study to be undertaken of the uptake of the labelled peptides into tumour-bearing mice.



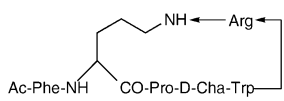
(**61**)

A series of cyclic lactam-bridged BH3 domains *e.g.* (**62**) have been synthesised[131] using a combined Fmoc/Bu^t/Bzl protection strategy, and were found to adopt highly helical structures, while a N-terminal to side-chain bridge as in the dynorphin

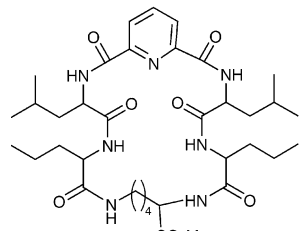
A (**1–11**)NH_2 analogue(**63**) was constructed[132] on-resin using PyBOP/HOBt/DIEA. This analogue became the first opioid peptide cyclised through the N-terminus that retained high opioid receptor activity. Cyclo (Val–Lys–Gly–Phe–Tyr) has been synthesised[133] and when tested, it stimulated macrophage bactericidal activity through non-opioid β-endorphin receptors. With a view to large scale synthesis, the highly potent anti-inflammatory drug Ac–Phe–[Orn–Pro–D-Cha–Trp–Arg] has been produced in the solution phase[134] by coupling two tripeptide fragments to give a linear hexapeptide precursor, followed by cyclisation to the cyclopentapeptide core using the BOP reagent. From two orthogonal libraries[135] utilising the four indispensable residues of the CXCR4 antagonist, T140, cyclo (Nal–Gly–D-Tyr–Arg–Arg) emerged as being equipotent with T140, while cyclo (His–D-Phe–Arg–Trp Aoa) exhibited[136] 5-fold higher potency at hMC-4R than that of α-MSH. [Aoa = 8-aminooctanoic acid]. The ionophoric activity of nature's enniatins and valinomycins has influenced[137] the design of (**64**) as a possible cytotoxic and ionophoric molecule. Conventional solution phase couplings such as the mixed anhydride and azide methodology were used in its synthesis.



(**62**)



(**63**)



(**64**)

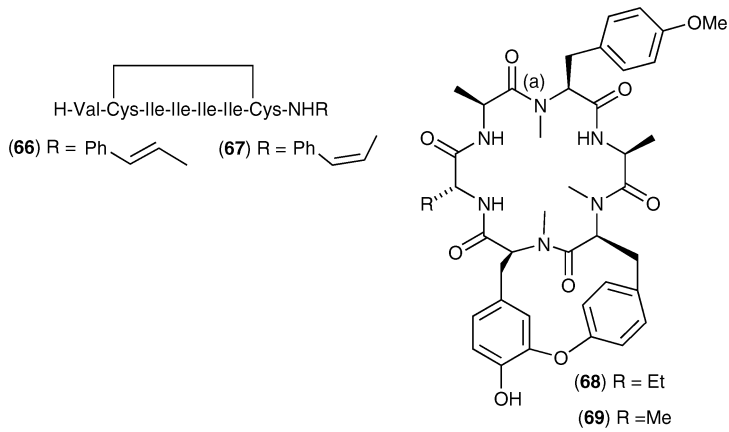
Consecutive macrocyclic pentapeptides such as (**65**, $n = 2$ or 3) have been shown[138] to form 3-turn and 4-turn α-helices which are stable in water and resist protein denaturing conditions, while cyclic metallopentapeptide modules, such as Pd(en)-$(MeHisXXXMeHis)^{2+}$ based on complexing with a methylated histidine can mimic an α-helical turn.[139] The use of peptide/metal-ligand hybrids for metal-assisted stabilisation of microstructures has been reviewed.[140] An evaluation[141] has been carried out of the β-turn mimetic properties of metal complexes of chiral pentaza-crowns, derived from reduction of cyclic pentapeptides, and reveal that if limited to three peptide chains, there is a reasonable chance that the molecules might show receptor recognition. Cyclo $(Gln)_5$ forms a nanotube[142] which has been the subject of further theoretical prediction and atomic force microscope observations. An alternative library of retro inverso peptides such as cyclo (Gly–L–Nal–D-Arg–D-Arg–D-Tyr) has been constructed[143] to look for leads based on FC 131.

(**65**)

2.6 Cyclohexapeptides

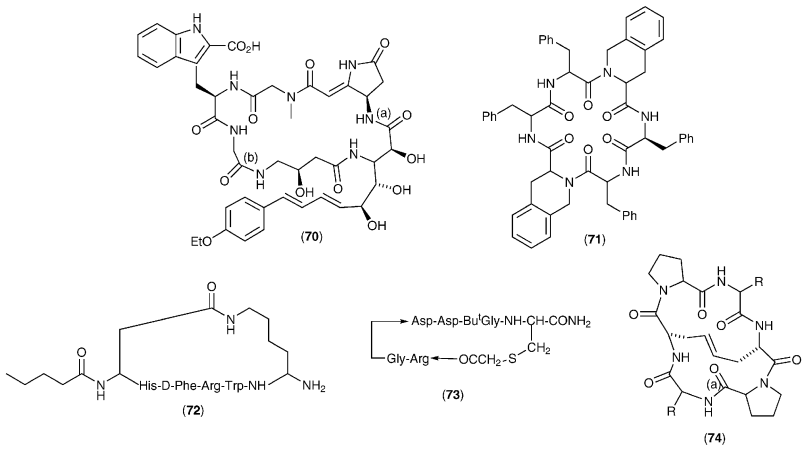
Microcionamides A (**66**) and B (**67**) have been sourced[144] from the Philippine sponge *Clathria (Thalysias) abietina*, and have significant cytotoxicity against human breast tumour cell lines and *Mycobacterium tuberculosis* $H_{37}Ra$. Of the three cyclohexapeptides, diandrines A, cyclo (Gly–Pro–Trp–Pro–Tyr–Phe), C, cyclo (Gly–Gly–Pro–Tyr–Trp–Pro) and D, cyclo (Gly–Gly–Pro–Tyr–Trp–Pro), isolated[145] from *Drymaria diandra*, only A showed a selective inhibitory effect on collagen-induced platelet aggregation (IC_{50} 44.2), while C and D are stable conformational isomers due to rotational isomerism around the Pro residues. The family of bicyclic hexapeptides extracted[146] from *Rubia cordifolia* has been augmented by another

congener, RA-XVII (**68**), and is in essence [D-Abu]^1-deoxybouvardin. Although the Dbu residue has little effect on conformation, cytotoxic activity was decreased. A triazole cis-amide bond surrogate has been fixed[147] into position (a) of RA-VII (**69**), but showed no cytotoxic activity, while three epimers of RA-VII (**69**) have been formed[148] *via* formation of oxazoles from thioamides or thioimidates followed by hydrolysis. The epimers, [Ala]^1-, [D-Ala]^2- and [D-Ala]^4-RA-VII showed very weak cytotoxicity, and from X-ray data and NMR work showed different conformations to that of the active conformation of (**69**). Of the three analogues,[149] [Gly]^1-, [Gly]^2- and [Gly]^4-RA-VII, the first analogue showed the highest cytotoxic activity, and it possessed a similar conformational profile to the natural RA-VII.

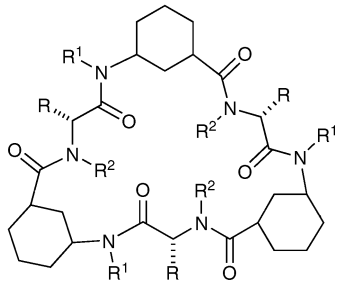


The demanding task of total synthesis of microsclerodermin E (**70**) has been achieved[150] for the first time starting with four fragments, carrying out a critical peptide coupling at position (a) in (**70**), with the macrocyclisation (40%) at position (b) being carried out using DPPA for 14 days. Three key components required in the synthesis of microsclerodermins have also been prepared.[151] Three cyclohexapeptides based on the antibiotic loloatin C have been synthesised[152] using both Fmoc-based solid phase and activated ester-based solution phase. These peptides, cyclo (Val–Orn–D-Phe–Asn–Asp–Trp), cyclo (Val–Orn–Leu–Trp–D-Phe–Asn) and Cyclo (Orn–Leu–D-Tyr–Pro–Trp–D-Phe) were confirmed as having helix-like structures with γ-turns. Macrocycles designed[153] with C-2 symmetry [of which (**71**) is one of eight synthesised] have been produced as cyclohexapeptide scaffolds, and have been shown to trap Holliday junctions in bacteria. Trapping these junctions was achieved in a second generation[154] of lipophilic cyclohexapeptides which had increased hydrophilicity due to the incorporation of tyrosyl residues. The histidine residue in the potent but non-selective hMC4R agonist (**72**) has been systematically substituted[155] and two analogues, one with ClAtc instead of His, the other with Arg replaced by Cit as well, were both potent agonists and more selective towards the hMC4R receptor. A three-dimensional orthogonal protection strategy[156] based on the Fmoc protocol has given pure samples of cyclo (Leu–Phe–Val–Ser–Cys–Asn) (cycloNL-6), which inhibits the action of matrix metalloproteinase 1 against progelatinase A, and solid phase protocols followed by cyclisation in the solution phase have provided[157] thioether-containing analogues such as (**73**) with good $\alpha_v\beta_3$ and $\alpha_5\beta_1$ receptor affinity. A number of cyclic analogues of the Arg/Trp-rich sequence Ac-RRWWRF-NH_2 have been prepared[158] and tested for their antimicrobial activity. Cyclisation induced high antimicrobial activity, with three adjacent aromatic residues giving the most pronounced effect, which underlined the importance of amphipathicity and the formation of hydrophobic clusters as being favourable for activity and selectivity. Previous work on cyclotetrapeptide hydroxamic acid inhibitors of HDAC6 has been extended[159] to include cyclohexapeptide analogues,

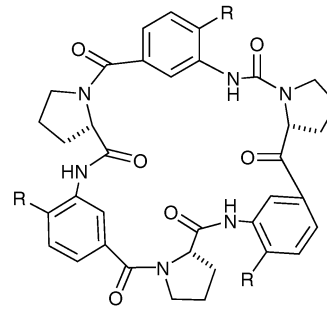
cyclo [Ser–Asp–Lys(Ac)–Thr–Ile–Gly] and cyclo [Ser–Asp–Asu(NHOH)–Thr–Ile–Gly]. Each was synthesised from Fmoc-Gly loaded resin, followed by cyclisation off-resin using HATU for activation. The hydroxamic acid cyclohexapeptide analogue had less activity than its cyclotetrapeptide counterpart, explained by the conformational differences reducing the binding capacity. Transposing structural elements of the somatotropin release inhibitory factor SRIF-14 onto a cyclohexapeptide template, fostered[160] the synthesis of a template, of which SOM 230, cyclo [(diaminopentylcarbamoyl)-HyPro–Phg–D-Trp-Lys–Tyr(Bzl)–Phe] showed the best therapeutic potential, graduating into phase I clinical trials. Macrocyclisation of the linear precursors to form the templates occurred at the Tyr(Bzl)-Phe bond using DPPA. Of eight linear hexapeptides comprising sterically-hindered Aib residues and turn-promoting Gly and Pro, only three could be cyclised[161] to form cyclo (Aib–Aib–Phe–Pro–Aib–Gly) and cyclo (Aib–Aib–Gly–Aib–Pro–Gly). DEPBT, PyAOP and DEPC proved to be the best macrocylisation agents. The conformation of the former was shown by X-ray crystallography to be two β-turns stabilised by H-bonding between Gly and Phe. The bicyclic compound (**74**) was constructed[162] in two stages, the first a ring-closing metathesis reaction to form the bridge followed by macrocyclisation at point (a) using HBTU/HOBt.



Cyclopeptides (**75**) and (**76**) represent new members to a family of self-assembly peptides[163] which exist as dimers with anti-parallel peptide rings linked by β-sheet H-bonds. C_3-Symmetric (**77**) with R = H, OMe, COOMe or COOBzl has shown potential[164] for chiral recognition of *N,N,N*-trimethyl-1-phenylethyl ammonium cations, while studies[165] on cyclo (D-Ala–Pro–Ala)$_2$ using CD and differential scanning calorimetry have shown that the cyclopeptide has a tendency to form channels inside lipid bilayers. Attenuated total reflection Fourier transform infrared spectroscopy and force field calculations[166] have been applied to interactions between cyclic lysine-containing peptides with hydrolysed silicon surfaces and with immobilised model peptides. The positively charged lysine side chains interact with OH groups on the silicon surface, whilst distinct differences in the bonding behaviour were seen with the various immobilised peptides. Cyclic hexapeptides continue to be a popular model for conformational analysis by the usual armoury of physical methods. Thus the epitope Asp–Pro–Val–Gly representing sequence 276–284 of glycoprotein D of Herpes simplex virus, when contained as a cyclic core in H–Ser–Ala–Leu–Leu–cyclo(Glu–Asp–Pro–Val–Gly–Lys)–NH_2 has been shown to possess a type I β-turn structure,[167] and applying a dipole intreraction model to CD spectra[168] has shown that in solution, cyclo (Gly–Pro–Gly)$_2$ interconverts between β- and γ-turns. An NMR study[169] has shown that the backbone conformation of a series of Trp-containing cyclohexapeptides has a significant effect on tryptophan fluorescence.



(**75**) R^1 = H, R^2 = Me
(**76**) R^1 = Me, R^2 = H

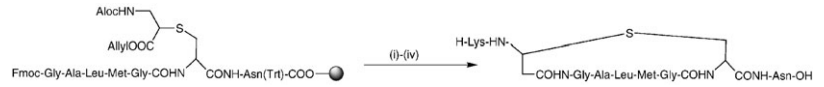


(**77**)

2.7 Cycloheptapeptides

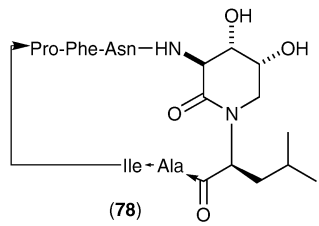
The marine fungus *Scytalidium* sp. has yielded[170] the moderately cytotoxic scytalidamides A, cyclo (Phe–MePhe–Phe–Leu–Pro–MeLeu–Aib) and B, which has the Pro residue replaced by 3-MePro. The sponge *Phakellia fusca Thiele* is also a source of cytotoxic (ED_{50} $<10^{-2}$ μg/mL) phakellistatin 13 whose structure has been shown[171] to be cyclo (Pro–Phe–Gly–Pro–Thr–Leu–Trp), while the seeds of *Goniothalamus leiocarpus* (Annonaceae) have been found to contain[172] leiocyclocin C, cyclo (Gly–Leu–Pro–Gly–Phe–Tyr–Pro) and a cycloctapeptide leiocyclocin D, cyclo (Gly–Ser–Pro–Tyr–Gly–Tyr–Pro–Pro). *Annona cherimola* produced[173] cherimolacyclopeptide C, cyclo (Pro–Gly–Ala–Ala–Trp–Ile–Pro) with *in vitro* cytotoxic activity of IC_{50} 0.072 μM against KB cells.

Yunnanins A, cyclo(Gly–Gly–Pro–Phe–Pro–Gly–Tyr) and C, cyclo (Tyr–Ser–Pro–Gly–Ile–Gly–Phe) from roots of *Stellarai yunnanensis*, together with phakellistatins 1, cyclo (Pro–Tyr–Pro–Ile–Pro–Ile–Phe) and 10, cyclo(Pro–Leu–Thr–Pro–Ile–Pro–Trp–Val) from the *Phakellia* sponge, have been synthesised[174] using Fmoc/Bu^t chemistry on 2-chlorotritiyl chloride resin, and using HATU for the macrocyclisation stages. Both yunnanins were confirmed to have *trans* conformations at the prolines, while phakellistatin 1 showed *cis* conformation at all prolines. Phakellistatin 10 was proved to have the all *trans* –Pro conformations, and interestingly the synthetic compounds produced although structurally identical to the natural products did not give the same cytotoxic properties a trend already reported on by Pettit and his group. Scytalidamide A (cyclo Phe–MePhe–Phe^3–Leu–Pro–MeLeu–Aib) mentioned earlier has been synthesised for the first time[175] on solid phase using two different linker resins. In one routine a Phe-silane resin was initially attached to Phe^3 with cyclisation using PyBOP (5eq) at the carboxyl of Phe^3, while the other approach utilised a backbone linker attached between MePhe and Phe^3 and the same cyclisation. The latter protocol gave the higher yield. A piperidone Ser–Leu surrogate inserted into stylostatin 1 as shown in (**78**) was incorporated[176] most efficiently by cyclisation at the Pro–Phe bond, but the surrogates failed to maintain the anti-cancer activity of the parent compound. Anchoring the β-hydroxyl group of Ser to a Wang resin has also proved a convenient way of on-resin synthesis[177] of stylostatin 1, using the allyl protecting group to cover the C-terminus while the residues were assembled using Fmoc/Bu^t protocols. The photoresponsive integrin ligand, cyclo (Lys–Ala–Arg–Gly–Asp–D-Phe–Val–AMPB) has been synthesised[178] by backbone cyclisation, and surface plasmon enhanced fluorescence spectroscopy showed that the *trans* azobenzene isomer had a higher affinity for the $\alpha_v\beta_3$ integrin than the *cis* form due to conformational preferences. As summarised in Scheme 13, two diastereomeric analogues of the ring C of nisin have



Scheme 13 Reagents (i) $Pd(PPh_3)_4$, AcOH, NMM (ii) PyAOP, HOAt, Pr_2^iEtN (iii) Fmoc-Lys-OH, DIC/HOBt (iv) TFA, H_2O, Et_3SiH.

been constructed on solid phase[179] using a triply orthogonal strategy, and analysed for identification/conformational purposes. Solid phase synthesis[180] based on 'cleavage by cyclisation' using Kenner's safety catch linker has worked well in the synthesis of polymyxin B-1 and analogues. Analogues in which the 6-methyloctanoic acid was replaced by shorter acyl changes still showed antibacterial activity, but any changes at the D-Phe–Leu segment gave loss of activity.



(**78**)

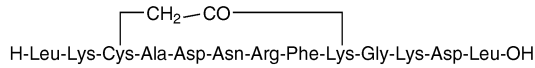
LC-MS has been used in sequencing bacitracin A and related minor components,[181] and in scanning water samples in SW Finland[182] and in Ireland[183] for microcystins and nodularin.

2.8 Cyclooctapeptides

Quite a few structures in this category have been found in natural sources. Thus the marine sponge *Axinella carteri* is a source[184] of cyclonellin, cyclo (Arg–Tyr–Pro–Tyr–Thr–Ala–Asn–Pro), whilst the aerial part of *Schnabelia tetradonta* (Sun) has yielded[185] schnabepeptide B, cyclo (Trp–Gly–Leu–Gly–Pro–Pro–Leu–Pro). The seeds of *Annona cherimola* have yielded[186] cherimolacyclopeptides A, cyclo (Pro–Gln–Thr–Gly–Met–Leu–Pro–Ile) and B, cyclo ((Pro–Gln–Thr–Gly–Mso–Leu–Pro–Ile), [Mso = Met oxide] with structures containing two β-turns and a new type of β-bulge. Squamtin A was found[187] in *Annona squamosa* and deduced to be cyclo (Val–Thr–Gly–Tyr–Mso–Pro–Ile–Ala). Three new cyclooctapeptides microtoenins A–C have been found [188] in the stems of *Microtoena prainiana* and given the structures cyclo (Ala–Val–Pro–Tyr–Leu-–Val–Pro–Phe), cyclo (Phe–Phe–Val–Pro–Phe–Gly–Ala–Ala) and cyclo (Pro–Tyr–Asn–Phe–Pro–Leu–Pro–Ile), respectively. Brachystemin C from the roots of *Brachystemma calycinum* has been shown to have[189] three β-turns in its cyclo (Tyr–Pro–Pro–Ile–Gly–Val–Ala–Ala) structure. Pseudostellarin G, cyclo (Pro–Phe–Ser–Phe–Gly–Pro–Leu–Ala) has been synthesised[190] in the solution phase, using a *p*-nitrophenyl ester at the Ala carboxyl for cyclisation.

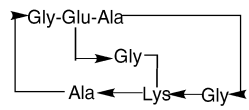
All four configurations, (2*S*, 4*R*),(2*S*, 4*S*), (2*R*, 4*S*) and (2*R*, 4*R*) of 4-NH_2MePro have been inserted[191] into the CLX sequence isolated from flax seed, cyclo (Pro–Pro–Phe–Phe–Ile–Leu–Leu–4-NH_2MePro). All of the diasteroisomeric forms showed immunosuppressive activity, which was lower than cyclolinopeptide A and cyclosporin A, and in the (2*S* 4*R*) analogue which was analysed by 2D-NMR, all amide bonds were *trans* with two loops similar to P-turns of type IV being present. Substitution of Cys or Hcy in position 11 and chloroacetylated Lys in position 18 of the 9–22 sequence, LKMADPNRFRGKDL of glycoprotein D of Herpes simplex virus type 1, has given cyclic thioether peptides,[192] such as (**79**), but their reactivity was lower than their linear analogues. Systematic insertions of

N^{β}-methylated aminoglycine (Agl) into the somatostatin analogue, cyclo (Cys^3–Phe^6–Phe^7–D-Trp^8–Lys^9–Thr^{10}–Phe^{11}–Cys^{14}) [SRIF numbering] revealed[193] that substitutions for residues 6 and 7 together with changes in configuration increased selectivity for the sst_4 receptor. Replacing D-Trp^8 systematically with stereoisomers of β-methyl-3-(2-naphthyl)alanine gave the L-threo-β-methyl 2-Nal as being the most selective[194] for sst_4. The same research group[195] have rationalised a 3D consensus structure at the binding pocket of the sst_4 which requires a unique set of distances between an indole/2-naphthyl ring, a lysine side-chain and another aromatic ring.



(**79**)

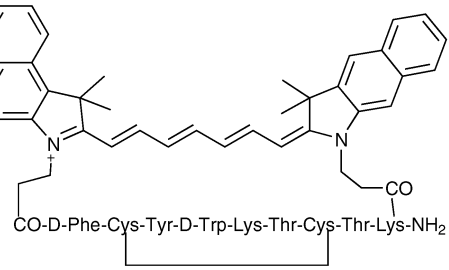
High level calculations[196] of amide protons in a calcium complex of the bicyclic peptide (**80**) gave results in good agreement with experimental NMR values, and a molecular dynamics investigation[197] of a nanotube comprising of cyclo (Trp–D–Leu–Trp–D-Leu–Trp–D-Leu–Gln–D-Leu) embedded in a dimyristoylphosphatidylcholine bilayer confirmed that it had conserved its hollow tubular structure. The geometries of cyclo [(D-AmP–L-AmP)$_4$], where AmP = α-amino pentanoic acid, and cyclo [(D-Ala–Phe)$_4$] have been worked out using DFT and ONIOM methods,[198] with the latter taking less computing time.



(**80**)

2.9 Cyclononapeptides

The cyclic nonapeptide moiety of chlorofusin (the core without its chromophore), cyclo (Thr–Ala–Asn^3–D–Asn^4–D-Leu–Thr–D-Leu–D-Ade–Orn), has been synthesized[199] both in this configuration and as its D-Asn^3–Asn^4 analogue. It was the NMR features of the former that reflected best the data for the natural product. In these syntheses macrocyclisation was carried out at the 4–5 position using EDCI/HOAt. Within another research group[200] the natural configuration of the cyclic core of chlorofusin was synthesised on solid phase by side chain immobilisation of Fmoc-Asp-Dmab on a Rink amide MBHA resin with final on-resin macrocyclisation at the 3–4 link. The cyclic peptide without its chromophoric side-chain does not inhibit p53/mdm2 interaction. Analogues with N-benzyl glycine systematically replacing the two Phe residues in cyclolinopeptide, cyclo (Leu–Ile–Ile–Leu–Val–Pro–Pro–Phe–Phe) have been synthesised[201] on solid phase with TBTU used for cyclisation. Modelling studies carried out on the analogues support the importance of edge-to face interactions between the Phe aromatic rings for the biological activity of these compounds. In order to selectively deliver optical probes into tumour sites, conjugating near IR-fluorescent compounds to bioactive peptides has proved successful as shown by the synthesis[202] of (**81**) based on the somatostatin analogue, octreotate. Structure-activity studies[203] have been carried out on the disulfide-linked cyclo (Cys–Leu^2–Leu–Arg–Met–Arg^6–Ser–Ile–Cys), that binds to intracellular adhesion molecule-1 (ICAM-1). On relating conformational/activity changes during the systematic replacement of residues by Ala, it is concluded that the segment from Leu^2-Arg^6 is functionally active and contains a β-turn over residues 4–7. This β-turn is retained in the more active (6-fold improvement) analogue with Lys at position 6 and Ala at 8.

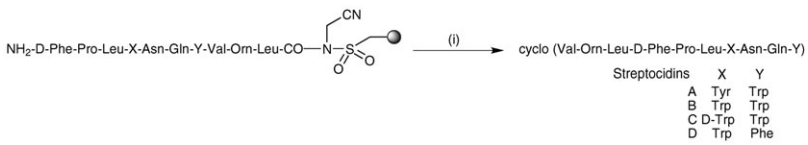


(**81**)

2.10 Cyclodecapeptides and higher cyclic peptides

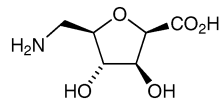
The only novel structure from nature that could be found under this category was the discovery[204] in trace amounts of phakellistatin 12, cyclo (Ile–Phe–Thr–Leu–Pro–Pro–Tyr–Ile–Pro–Pro) in the sponge *Phakellia* sp. It has a cancer cell growth inhibitory ED_{50} of 2.8 μg/mL. The tendency for linear precursors of amphipathic antimicrobial cyclic peptides such as tyrocidine A to pre-organise for cyclisation has been made use of in the synthesis[205] of streptocidins A–D using the traceless cyclisation (safety-catch principle) on-resin as summarised in Scheme 14. The same approach[206] has been used to systematically replace all positions in tyrocidine A, cyclo (Leu–Orn–Val–Tyr–Gln–Asn–D-Phe–Phe–Pro–D-Phe) with Ala, and on conformational analysis all analogues seem to preserve a common conformation seen in the parent compound. Substitution of Gln in tyrocidine A with a cationic amino acid led to a 140-fold enhancement in therapeutic index. A 192-member library of antibacterial cyclic decapeptides was successfully constructed[207] using the Scheme 14 approach.

The cyclisation protocol summarised in Scheme 14 was also relevant[208] for the synthesis of gramicidin S, cyclo (D-Phe–Pro–Val–Orn–Leu)$_2$ and analogues, this approach proving more efficient than the chemoenzymatic approach[209] using gramicidin thioesterase with an immobilised linear decapeptide. Cyclisation of H-D-Phe–Pro–Val–Orn–Leu-oxime resin, without protection of the ornithinyl side chain produced semi-gramicidin S (cyclic monomer) and gramicidin S in 9 and 40% yields respectively.[210] The Kaiser oxime resin approach was also used[211] in substituting the Pro residues in gramicidin S with (2*S*, 4*R*)-and (2*S*, 4*S*)-4-amino-Pro, to give products with marked permeabilising activity on the outer membrane of Gram negative bacteria. When the furanoid sugar amino acid (**82**) was incorporated[212] between Leu and Val in gramicidin S a novel hexameric β-barrel-like structure was observed, while the X-ray data[213] from trichloroacetylated and bromobenzoylated ornithinyl analogues of gramicidin S confirmed the usual sets of antiparallel pleated β-sheets and type II' β-turns. An FT-IR study[214] of gramicidin S with metal ions, Li^+, Na^+, K^+, Rb^+ and Cs^+ has shown that the cation polarisabilities are the result of fast fluctuation of the cations between four or six CO groups of the peptide backbone. Modifications have been carried out[215] to a designer 14-residues analogue of gramicidin S [cyclo (Val–Lys–Leu–Lys^4–Val–D-Tyr–Pro–Leu–Lys–Val–Lys–Leu-D–Tyr–Pro)] by varying the residues in position 4. All of the



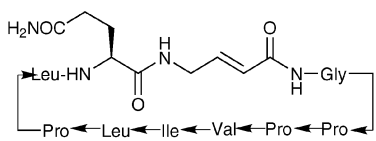
Scheme 14 Reagent: 20% DIPEA/THF.

L-residue substitutions made, resulted in poor antimicrobial activity, while the activity of the D-substitutions tended to improve based on the hydrophilicity of the residue inserted, with D-Lys showing the best therapeutic index. Conformational preferences of gramicidin S have been worked out[216] using ion-mobility mass spectrometry.

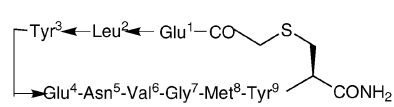


(**82**)

In a series of disulfide-bridged cyclodecapeptides designed[217] to inhibit the transcription factor activator protein-1 (AP-1), the most potent inhibition was exhibited by Ac-cyclo (Cys–Gly–Gln–Leu–Asp–Leu–Ala–Asp–Gly–Cys) with an IC_{50} = 8 μM. A cyclic olefine format (**83**) has been used[218] to restrict the conformation of the substrate sequence of human T-cell leukaemia virus type-1 (HTLV-1). Initially the side chain of Glu(Gln) was linked to a CLEAR resin *via* labile linker and the olefine bond constructed on-resin from an aldehyde using the Horners-Emmons reaction, with the macrocyclisation mediated by EDC/HOAt at the Gly residue. Analogue (**83**) functioned as a competitive inhibitor of HTLV-1 protease. By choosing two of the ten residues of a series of cyclic decapeptides, to be Pro or N-substituted residues at the *i* and *i* + 4 positions, the β-sheet structure can be fixed,[219] and by further disposition of residues the conformation can be fine-tuned to have four residues pointing one way, or two pointing in an opposite manner. The non-phosphorus containing inhibitor (**84**) with high-affinity binding to the Grb2-SH2 domain has been subject[220] to an SAR study, which found the analogue with substitutions Gla^1, $Phe(OMe)^2$, Ach^4, NPG^8, $Phe(4\text{-}NH_2)^9$ and *R*-$Cys(O)^{10}$ to be a potent antagonist (IC_{50} = 0.026 μM). The cyclodecapeptide cyclo (Gly–Met–Thr–Cys–Ser–Gly–Cys–Ser–Arg–Pro) has been shown[221] to bind soft metals selectively, and is a good model of the binding loop of the copper metallochaperone Atx1.



(**83**)

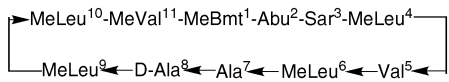


(**84**)

NMR Experiments combined with molecular simulation with X-PLOR have been employed[222] to determine the solution conformation of loloatin C, cyclo (Val–Orn–Leu–D-Tyr–Pro–Trp–D-Phe–Asn–Asp–Trp), in three different solvent systems. In DMSO an inverse turn structure is stable, but is de-stabilised in TFE to a dumb-bell structure, with all the hydrophobic side chains pointing upwards, and the hydrophilic side chains projecting to the other side together with most carbonyl oxygens. This amphiphilicity is of interest in relation to loloatin C's antibiotic activity. Infrared photon echo experiments[223] have explored the H-bond connectivities in antamanide, cyclo (Val–Pro–Pro–Ala–Phe–Phe–Pro–Pro–Phe–Phe), and an *in silico* molecular dynamics study[224] on cyclo (NNNL——KDT), where the middle section ranged from YNGK to KGT, showed promise as a means of selecting peptide-vaccine candidates with full biological activity.

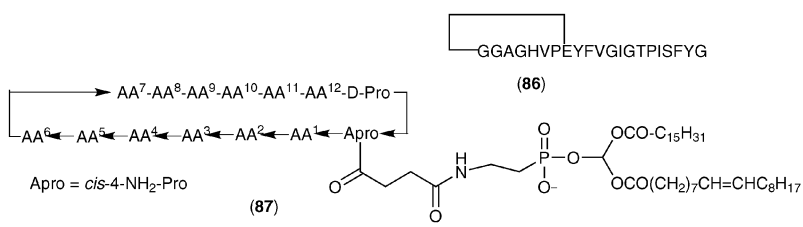
A number of publications describe work on the immunosuppressive cyclosporins. In an extensive and demanding synthetic challenge[225] pseudo-proline residues have been inserted into the 4–5 positions in cyclosporin A (**85**), and their conformations determined by NMR. Insertion of Ψ^{MeMe}Pro at position 5 maintains binding to cyclophilin A and to calcineurin, and possesses a *cis* amide at 5–6, while all other peptide bonds are *trans*. Similar insertions[226] into cyclosporin C, reduced the

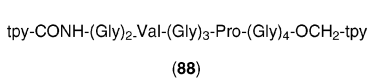
number of conformations, induced a *cis* amide at the 1–2 position resulting in complete loss of binding to cyclophilin A. Non-immunosuppressive cyclophilin-binding cyclosporin A derivatives have been produced[227] by insertion of various alkylthio side chains at the Sar^3 position in (**85**). Amongst the best analogues which displayed potent *in vitro* anti-HIV-1 (IC_{50} = 46 nM) were [2-(Me_2- or Et_2-amino) – ethylthio-$Sar]^3$,[(4′-OH)$MeLeu]^4$-(**85**) which also showed low immunosuppressive activities. As $[MeVal]^6$-(**85**) was known to be non-immunosuppressant, further analogues modified at positions 4 and 8 showed similar properties,[228] which inferred that binding to cyclophilin and/or inhibiting rotamase activity may be a necessity for neutrophic effects. Two cyclosporin analogues, (7-phenyl)(7-desmethyl)-(**85**) and its 6,7 dihydro analogue, out of eight produced[229] showed *in vitro* anti-parasitic effects against *Trypanosoma cruzi* with IC_{50} values of 0.82 and 3.41 μM, respectively. Crystal structures of cyclosporin G, [$norVal^2$-(**85**)] and cyclosporin H, [D-$MeVal^{11}$-(**85**)] have shown[230] that small local changes can be associated with significant structural transformation in the molecules.

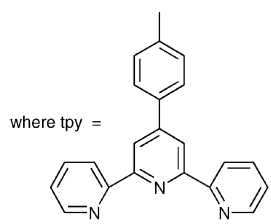


(**85**)

Thus cyclosporin G has a novel cloverleaf motif with no H-bonded structure, and its 9–11 *cis* peptide bond has moved to 11–1, while in cyclosporin H the bonds are all *trans* and it has a convoluted conformation rather than β-sheet. Evidence accumulated from a NMR/CD study[231] of metal-ion interaction with cyclosporin A (**85**) supports the possibility that (**85**) has ionophoric properties for biologically important metal ions. Cyclosporin A has also been used[232] as a 'guinea-pig' molecule to correlate fast and slow spinning sidebands in solid-state NMR experiments, and the $MeBmt^1$ residue in (**85**) has been synthesised[233] stereoselectively. To increase the bioavailability of cyclosporin A, dipeptides have been linked[234] to the hydroxy group of $MeBmt^1$, which at physiological pH can cyclise *via* dioxopiperazine formation to release the parent molecule, thus functioning as a pro-drug.





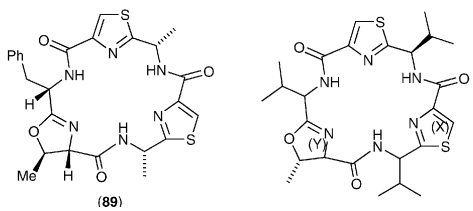


Tritium-labelled cyclosporin A has been obtained[235] using a metal catalysed H-isotope exchange procedure. In an effort to disengage the need to form a cyclophilin-cyclosporin calcineurin complex to achieve immunosuppression, α-substitution at the Sar^3 residue, with *e.g.* SMe has resulted[236] in direct calcineurin inhibition and rapid cyclophilin 18 binding. An auxiliary 1-phenyl-2-mercaptoethyl derivative on the N-terminal Gly residue[237] in association with a thioester group has been used to

cyclise the linear precursor of cyclo (Gly–Ser–Pro–Tyr–Ser–Ser–Asp–Thr–Thr–Pro–Ala). No protection of other side chains was needed. Microcin J25, a potent antibacterial, was known to have thermal stability not readily explained by a head-to-tail cyclised backbone originally put forward in 1999. New NMR data[238] suggests that the thermal stability can be explained by a side-chain-to backbone link between the N-terminal amino group and the carboxyl of Glu^8 cyclisation as shown in (**86**), but with the C-terminal residues threaded through the ring in a noose-like conformation. A library[239] of 35 peptidomimetics based on (**87**) which scan the residues 444–489 of the subdomain III of the apical membrane antigen-I, has been produced by solid phase assembly of linear precursors which were then cyclised using HATU/HOAt at position 6–7. Metal-binding domains (tpy) at each end of an undecapeptide pre-disposed to cyclisation (**88**), in the presence of iron (II) salts form[240] cyclometallopeptides.

2.11 Peptides containing thiazole/oxazole rings

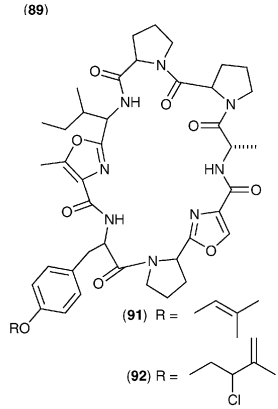
The ascidian *Didemnum molle* has been shown[241] to be a source of didmolamides A (**89**) and B, which appears to be a biosynthetic precursor of A where the oxazoline ring is replaced by threonine. An antifouling compound from the sponge *Haliclona* sp. has turned out[242] to be a sulfone of the already known waiakeamide. Bistratamides E–H from the ascidian *Lissoclinum bistratum* have been given the structures[243] listed under (**90**) while the I and J congeners are threonine precursors of the Me-oxazoline ring in (**90**). The sponge *Myriastra clavosa* has yielded[244] myriastramides A (**91**), B(**92**) and C, cyclo (Val–Oxz–Val–Pro–Pro–Val–Thz–Trp).



(**89**)

(**90**)

Bistratamide E X = thiazole, Y = Me, oxazoline
Bistratamide F X = oxazoline, Y = Me, oxazoline
Bistratamide G X = oxazole, Y = Me, oxazole
Bistratamide H X = thiazole, Y = Me,oxazole

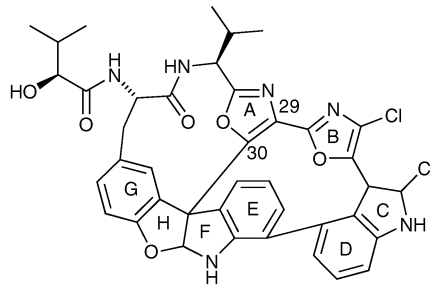


(**91**) R =

(**92**) R =

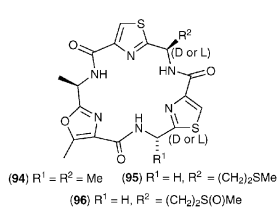
Recent advances in the total synthesis of oxazole-containing products have been reviewed.[245] The chronicle of the 5-year campaign to synthesise diazonamide A, both in its original presumed structure and in the now accepted structure (**93**) has been reported.[246] Details of the first synthesis of the new structure (**93**) have also appeared.[247] A second total synthesis of (**93**) from the same research group,[248,249] involved as key stages the macrocyclisation at C29–C30 using SmI_2 and an oxidation of an indoline to an oxindole using $Pd(OH)_2$. Some building blocks towards the diazonamide structure, requiring key coupling stages have also been explored such

as the coupling[250] between an arylstanane aminal and a palladium complex to link rings E to D, and the rhodium-catalysed synthesis of a C(3) substituted oxindole.[251]

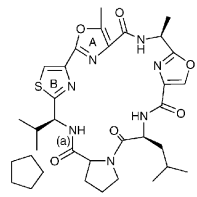


(**93**)

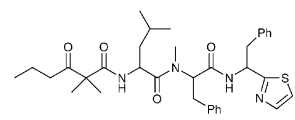
The availability of Fmoc-heterocyclic amino acid derivatives[252] has opened up a flexible solid-phase method to prepare diastereoisomeric precursors which can be cyclised using PyBOP/DMAP to the tenuecyclamides A or B (**94**) and C (**95**) and D (**96**). Full experimental details for the synthesis of mollamide, which first appeared as a preliminary communication in 1999, have been made available.[253] Leucamide (**97**) has been synthesised[254] from linear precursor with the heterocyclic rings already assembled. Ring A was constructed linked to B *via* a diethylaminosulfur trifluoride-mediated cyclisation of a β-hydroxyamide, with the final macrocyclisation at position (a) in (**97**) using HBTU/DIPEA (87% yield). A total synthesis[255] and an X-ray structure, has been carried out on dolastatin 18 (**98**) from *Dolabella auricularia*. The thiazole unit was available from another published work, and was added to sequentially with other units which included (*R*)-MePhe.



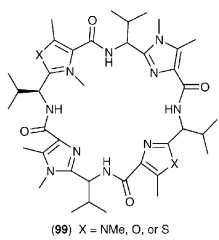
(**94**) $R^1 = R^2 = Me$ (**95**) $R^1 = H$, $R^2 = (CH_2)_2SMe$
(**96**) $R^1 = H$, $R^2 = (CH_2)_2S(O)Me$



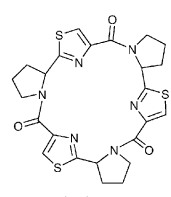
(**97**)



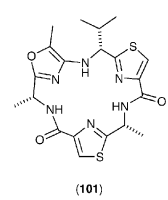
(**98**)



(**99**) X = NMe, O, or S



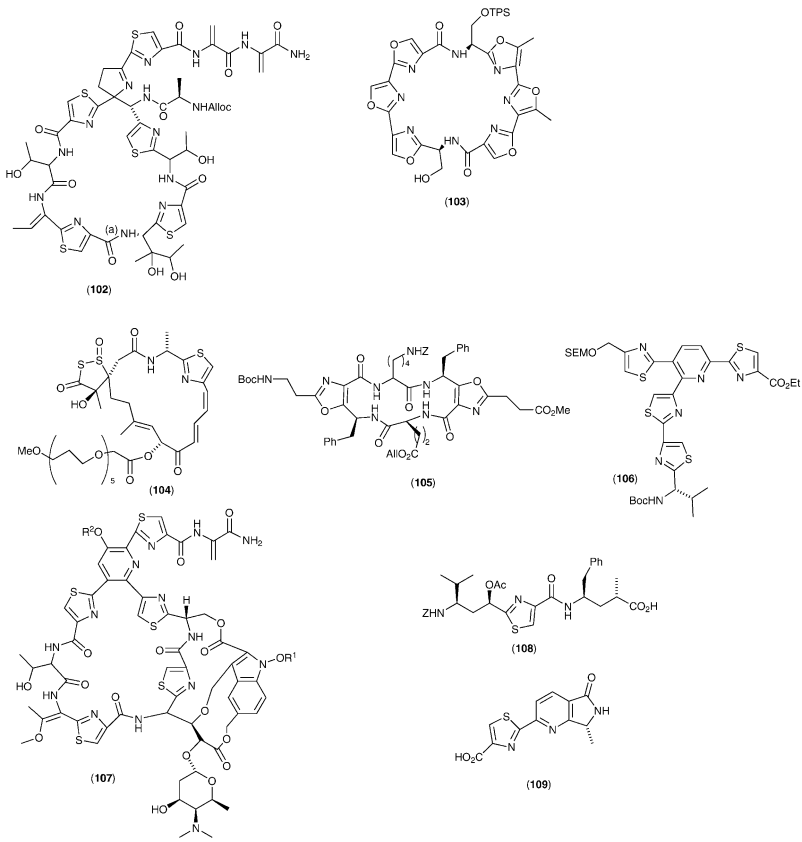
(**100**)



(**101**)

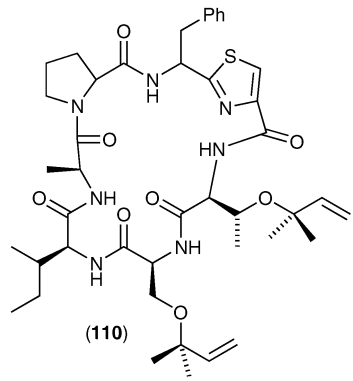
Full details have been made available[256] of the cyclooligomerisation of thiazole monomers to analogues of the Lissoclinum families of cyclic peptides. Imidazole analogues (**99**) of the same family series[257] have also been synthesised, macrocyclisation being carried out with FDDP(pentafluorophenyl diphenyl phosphinate)/DIPEA. The same cyclisation reagents (or DPPA) have been used[258] to cyclooligomerise Pro-thiazole combinations to give analogues such as (**100**). Highlights of a new synthesis[259] of dendroamide A (**101**) included the use of bis(triphenyl)oxodiphosphonium trifluoromethanesulfonate for the cyclodehydration of β-keto dipeptides to form oxazole rings and to form thiazole rings from cysteine dipeptides. Final cyclisation of the preformed heterocyclic amino acid linear precursor was enabled using PyBOP/DMAP in 81% yield. In the total synthesis[260] of *cis,cis*-ceratspongamide, cyclo (Ile–Oxz–Phe–Pro–Thz–Phe–Pro), a 4 + 3 fragment condensation was

carried out before macrocyclisation, followed by cyclodehydration to give the authentic compound, which has also been further examined[261] by X-ray crystallography. Many groups have progressed towards a total synthesis of some of the complicated structures characterised under this sub-section. In the 'almost there' category comes the impressive linking together of fragments of thiostrepton[262] to accomplish its thiazoline macrocycle (**102**), the result of cyclisation at position (a) in (**102**). Six oxazole units have been linked together prior to their cyclisation to (**103**) as part of the telomestatin synthesis,[263] while 2-bromo-5-hydroxy –Trp-oxazole, the α-keto-β-amino unit and the side chains of kermamide B have been synthesised.[264] Potent antitumour activity has been identified in C-8 ester derivatives such as (**104**) of leinamycin,[265] while A-ring modifications[266] to thiazole antibiotic GE2270A gave analogues with enhanced aqueous solubility.



Orthogonally-protected macrocyclic scaffolds such as (**105**) have been produced[267] by cyclisation of linear precursors using DPPA/$NaHCO_3$ at the Lys–Phe bond. The pyridine domain (**106**) within amythiamicin has been synthesised[268] using a Bohlmann-Rahtz reaction between a 1-(2-thiazolyl)propyn-1-one and an enamine. The anti-bacterial profile of the nocathiacins (**107**) is sufficiently interesting, that every effort is being pursued to improve their physical characteristics by derivatisation, *e.g.* alkyl,[269] phosphate and carbamate derivatives[270] at R^1 and R^2, and Michael addition products[271] to the side-chain dehydroalanine moiety. Most derivatives gave improved water solubilities without compromising anti-bacterial activity. α-Chiral 2-substituted 4-bromothiazoles have provided building blocks for fragments of GE2270 D2 and dolabellin.[272] The tubuvaline-tubuphenylalanine fragment (**108**) of the tubulysins has been constructed,[273] and the γ-lactam hydolysate (**109**) from

cyclothiazomycin has been synthesised[274] stereoselectively. Molecular modelling techniques[275] have been applied to cyclo (Aaa–Thz)$_3$, cyclo (Aaa–Oxz)$_3$, cyclo (Aaa–Thz)$_4$ and cyclo (Aaa–Oxz)$_4$ with the conclusion that the heterocyclic constraints could well be starting points for the design of novel artificial proteins and enzymes. Advanced NMR techniques and simulated annealing methods[276] have shown trunkamide A (**110**) to be a rigid molecule dominated by two trans-annular H-bonds and the volume of the dimethylallyl side-chains. The conformational differences offered by different enantiomers of Phe present in the macrocycle have served to explain why the L-Phe trunkamide A converts readily to the D-Phe analogue. Crystal data and CD studies on cyclo (Cha–Oxz–D-Val–Thz–Ile–Oxz–D-Val–Thz) a cyclohexylalanine-containing analogue of ascidiacyclamide have appeared contradictory,[277] in that the structure was defined as planar by the former method and folded by the latter. The conformation of virginiamycin M_1 in methanol or DMSO has been shown[278] to be different from its $CDCl_3$ form.



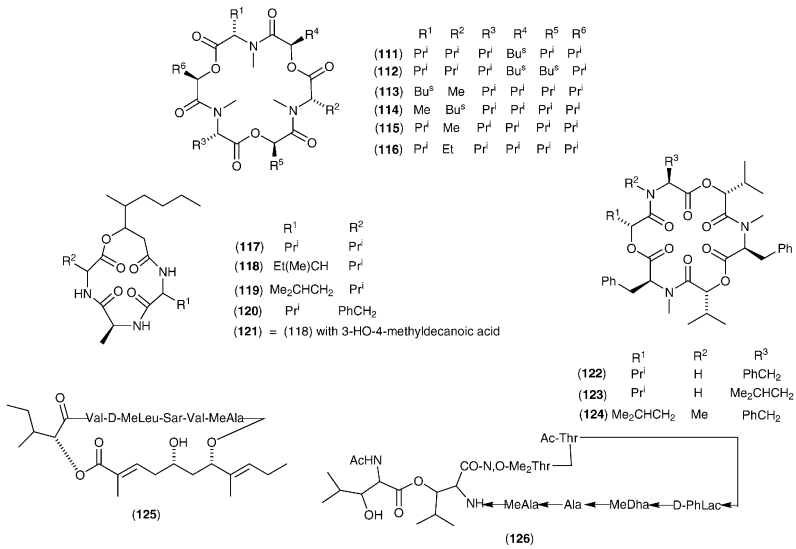
(**110**)

2.12 Cyclodepsipeptides

Ever since the start of these Specialist Periodical Reports, the cyclodepsipeptides have always generated significant interest and justified their own sub-section status. Again, a great deal of attention has to be given to these compounds, as nature has added significantly to the wealth of structures it produces. As some of the structures have now become 'page-fillers' in their complexity, it was good to see an attempt[279] being made to formalise an abbreviated system of representation. The most interesting aspects of the biological and medicinal properties of cyclodepsipeptides have been reviewed,[280] and the important developments in cryptophycin synthesis up to 2000 have also been described.[281]

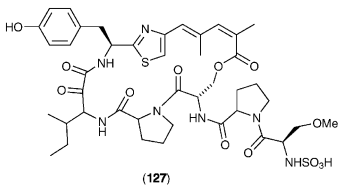
The insect pathogenic fungus *Verticillium hemipterigenum* have been shown to be a source[282] of enniatins H (**111**) and I (**112**), which strongly inhibited the human malaria parasite and the growth of mycobacteria. Four new enniatins, J_2(**113**), J_3(**114**), J_1(**115**) and K_1(**116**) have been found[283] in *Fusarium* sp. Strain F 31. Guineamides (A–F) have been given the structures,[284] cyclo (Mapa–Lac–MeVal–MePhe–Ala–Thz), cyclo (Maba–Hyiv–MeVal–Me Phe–Ala–Thz), cyclo (Maoya-Hmpa-*N,O* Me_2Tyr–Val–MeAla), cyclo (Maha–Hyiv–MeVal–Hyiv–D-*N,O*-Me_2-Tyr–Gly), cyclo (Dmhha–Hyiv–Ala–MeVal–Pro–D-MePhe–Gly) and cyclo (Dmhha–Val–MePhe–Pro–MePhe–Gly), respectively where Mapa = 2-methyl-3-aminopentanoic acid, Maba = 2-methyl-3-aminobutanoic acid, Dmhha = 2,2-dimethyl-3-hydroxyhexanoic acid, Maoya = 2-methyl-3-amino-oct-7-ynoic acid and Maha = 2-methyl-3-aminohaxanoic acid. A moderate cytotoxicity to a mouse neuroblastoma cell line was found in B and C. Beauveriolides (IV–VIII) from acetone extracts of *Beauveria* sp. FO-6979 have been allocated[285] the structures (**117–121**), while the new beauvericins (D-F) from Beauveria sp. FKI 1366 have

structures[286] (**122–124**). Full details[287] of the previously communicated structure of aurilide (**125**) from the sea hare *Dolobella auricularia* have appeared together with its improved total synthesis, and it has also been the subject of a solid phase library synthesis[288] (multipin technology-solution phase macrocyclisation with EDCI/HOAt) which gave related analogues as well. YM-254890, a novel G(q/11) inhibitor form *Chromobacterium* sp. QS3666 has been found to have[289] structure (**126**), which exists as a mixture of two conformers (isomerism around Phlac-MeΔAla) in solution. Natural analogues with different acyl groups on the hydroxy-leucic acid residue, and two analogues corresponding to the products of the hydrogenation of the dehydroalanine residue have also been studied.[290] Ulongapeptin, which is cytotoxic (IC_{50} = 0.63 mM), from the marine cyanobacterium *Lyngbya* sp. was characterised[291] as cyclo (Amo–Lac–MeVal–Val–D-MeVal–D-MePhe–Val), (Amo = 3-amino-2-methyl-7-octynoic acid), while the mycoparasitic *Cladobotryum* sp. yielded[292] two novel cytotoxic cyclodepsipeptides, cyclo [MePhe–Apa(or Aba)–Hyl–Pro–Phe–MeVal], where Hyl is leucic acid, Aba is γ-aminobutanoic acid and Apa is δ-aminopentanoic acid. Physical methods including X-ray diffraction has enabled[293] the structures of isaridins A and B to be confirmed as, cyclo (β-Gly–Hyl-Pro–Phe–MeVal–MePhe) and cyclo (β-Gly–Hyl–3–MePro–Phe–MeVal–MePhe) respectively. *Cis*-Amide bonds were observed at Hyl–Pro in A and Hyl-3-MePro in B.



Scleritodermin A, from the lithistid sponge *Scleritoderma nodosum* has been identified[294] as (**127**), while the sponge *Neamphius huxleyi* possessed[295] the HIV-inhibitory depsipeptide, neamphamide A (**128**), related to the callipeltins but with an expanded macrocyclic ring. Obvious similarities with aurilide (**125**) can be deduced for kulokekahilide-2 (**129**) isolated[296] from the mollusk *Philinopsis speciosa* which shows selective cytotoxicity. A strain of *Penicillium brevicompatum* from a sponge has yielded[297] petrosifungins A and B identified as cyclo [(*N*-Bz)Thr–Val–Pro–Pip–Pip] and cyclo [(*N*-4-OHBz)Thr–Val–Pro–Pip–Pip], respectively. From axenic cultures of *Scytonema hofmanni* PCC 7110, the known scyptolin A and B and the novel hofmannolin cyclo [(Hmv–Glu)Thr–Tyr–Ahp–OMeTyr–MeTyr–Val) with Hmv = 2-hydroxy-3-methylvaleric acid and Ahp = 3-amino-6-hydroxy-2-oxopiperidine, have been isolated.[298] Elastase and chymotrypsin inhibitors have been extracted[299] from the cyanobacterium *Planktothrix rubescens* and identified as planktopeptins BL1125, cyclo-[Ile–(Glyceric–Hty–Gln)Thr–Leu–Ahp–Thr–Me$_2$Tyr], BL843, cyclo-[Ile–(Glyceric–Leu–Gln)Thr–Leu–Ahp–Thr–Me$_2$Tyr] and BL1061, cyclo

[Ile–(pGln)Thr–Leu–Ahp–Thr–Me_2Tyr]. In two separate investigations on the cyanobacterium *Lyngbya Majuscula*, one has yielded[300] a higher homologue, homodolastatin 16 (**130**) of dolastatin 16, and the other[301] gave lyngbyastatin 3, cyclo [Gly–MeVal–Me_2Tyr–Ibu–MeAla–Amha–(2-OH-3-Me valeric acid)–Gly–Me-Leu], where Amha = 3-amino-2-methylhaxanoic acid and Ibu = 4-amino-2,2-dimethyl-3-oxopentanoic acid. By monitoring[302] the culture broth of *Paenibacillus* sp. for metabolites to inhibit lipopolysaccharide activity, it was possible to identify heptadepsin, cyclo [D-Ala-(GHHD)Ser–D-Val–Ile–D-Ala–Ser–D–Asn] as an active component, where GHHD = 17-guanidino-3-hydroxyheptadecanoic acid.



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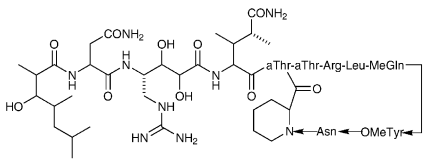
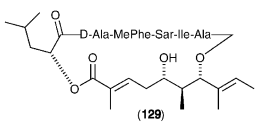
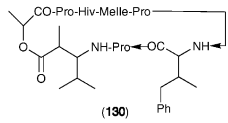


(**128**)

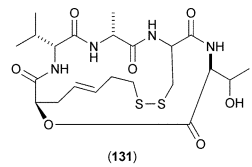


(**129**)



(**130**)

A record harvest of new structures identified, however, has not affected the desire to seek total syntheses of known cyclodepsipeptide structures. The first total synthesis[303] of FR-901375 (**131**) form *Pseudomonas chloroaphis* No. 2522 has been successful, starting form NH_2–D-Val–D-Val–D-Cys(Trt)–Thr(OTBS)–OH and following attachment of (3*R*, 4*E*)-3-hydroxy-7-mercapto-4-heptenoic acid, a mild Mitsunobu macrolactonisation closed the first ring, which was followed by the generation of the bisulfide link using iodine/methanol. The clinically-interesting antibiotic, ramoplanin A2 (**132**) (Hpg = hydroxyphenylglycine and ClHpg = chlorohydroxyphenylglycine) and its aglycone form have been synthesised[304] by initially incorporating the labile depside link into Boc–Leu–D-Ala–ClHpg-βOHAsn (Trt), using EDCI/DMAP. Two macrocyclisation sites, Phe–Orn and Gly–Leu, were explored using EDCI/HOAt with both giving successful cyclisation but a greater yield (89%) was achieved in the former case presumably due to some β-sheet preorganisation. To stabilise the structure for possible drug development, the depside link has been replaced[305] as an amide *via* the incorporation of 2,3-diaminopropionic acid for the hydroxyasparagine which gave a more potent analogue, and another analogue with an acetyl Asn^1 residue has also been produced. In the synthesis[306] of yanucamide (**133**), the depside link was formed using EDC/DMAP prior to the macrolactamisation step at position (a) in (**133**) which was carried out using BOPCl/NMM. The final macrolactonisation step in a new synthesis[307] of hapalosin (**134**), was carried out using activation of a 2-pyridylketone oxime ester at position (X) by $Cu(OTf)_2$ in what is described as a double activation step. Lipase-catalysed resolution of β-hydroxy-γ-amino and acid and the β-hydroxy acid, was a feature in another synthesis[308] of (**134**) which relied on macrolactamisation at position (Y). Based on the scaffold (**135**) four cyclic compounds have been synthesised[309] which represent core structural modifications of class B synergimycins. Macrocyclisation occurred at position (b), using HATU, TBTU PyBOP and /or DEPBT. Using a 'cut and paste' chemical approach,[310] the CO of the ester in the D-lactate analogue of HUB-7293 has been replaced by CH_2 to form ether analogues.



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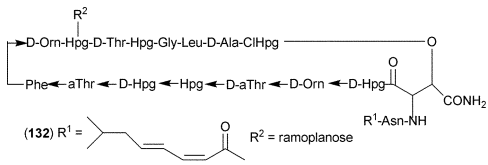

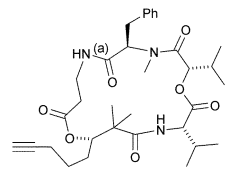

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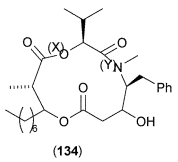

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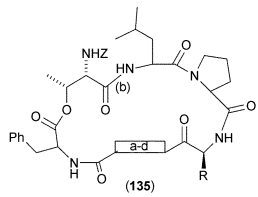

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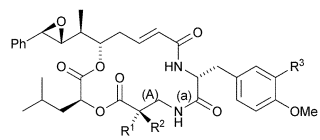
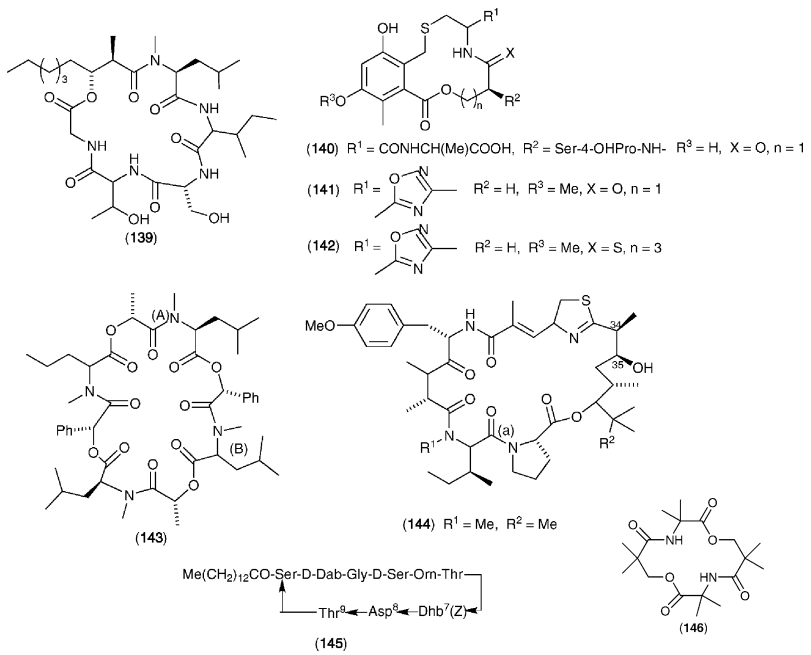


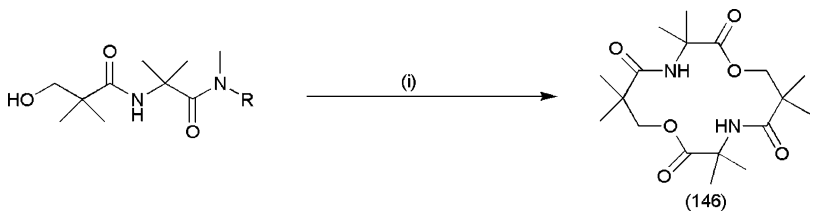
(136) cryptophycin B R^1 = H, R^2 = Me, R^3 = H
(137) arenastatin A $R^1 = R^2 = R^3$ = H
(138) cryptophycin 52 (LY355703) $R^1 = R^2$ = Me, R^3 = Cl

Although sparse in their amino acid content, the cytotoxic potency of the cryptophycins demand interest especially in the need for analogues that give higher stability towards *in vivo* hydrolysis. There have been many of these published already, but new ideas still appear, such as the 3-fragment approach to the synthesis[311] of cryptophycin B (**136**) and arenastatin A (**137**). Deoxy linear precursors were first constructed using asymmetrically-constructed fragments, followed by cyclisation at point (a) using 2,4,6-trichlorobenzoyl chloride/DMAP. Epoxidation was carried out as a final step using dimethyldioxirane. Two novel steps in another synthesis[312] of (**137**), included introducing the epoxide (using base-catalysed epoxidation from bromoformate) prior to cyclisation which took place using a ring-closing metathesis approach (Grubbs 1 catalyst). A really novel macrocyclisation step, which generates the residue (A) in the cryptophycin ring, has used[313] the acylating power of a β-lactam ring for the macrocyclisation stage. A highly enantioselective and convergent synthesis[314] of potent cryptophycin 52 (**138**), currently in clinical trials as an antimitotic tumour agent, was based on generating two tetrahydrofuran rings from 4-phenylbutyrolactone, and after linking the fragments to form a diester linear precursor macrocyclisation at point (a) utilised FDPP/DIPEA, followed by epoxidation using *m*-chloroperbenzoic acid. Structure-activity relationships[315] have been initiated around the styrene epoxide ring with the conclusion that substitution at *meta* or *para* positions of the phenyl ring in some cases can enhance potency. Activity of analogues of globomycin (**139**) has been derived from modifications[316] at MeLeu, Thr and the length of the fatty acid side-chain. Lengthening the latter by 4 carbons brings a 4 to 8-fold increase in potency, while *N*-methylation of Leu is essential to retain activity. By using a 2,3-epoxy ring as a stereo-controlling precursor for the synthesis[317] of the stevastelins, it was possible to achieve macrocyclisation in its presence, but attempts to open the ring subsequently failed. A series of enniatin B analogues based on the scaffold, cyclo (MeXaa–HyCar–MeIle–(*R*)–Lac–MeIle–(*R*)–Lac) (HyCar = hydroxy carboxylic acid) have been produced[318] to study their activity against the nematode *Haemonchus contortus Rudolphi* in sheep. Cyclisation of linear precursors occurred at the Lac-MeXaa bond using BOP-Cl and as result of X-ray analysis, correlation between activity and conformation was possible. A variety of analogues of the DNA gyrase inhibitor cyclothialidine (**140**) have been synthesised[319] with the conclusion drawn that an increase of lipophilicity

had a positive impact. The most active amongst the analogues were (**141**) and (**142**). Insertion of small-ring conformational restrictors at points (A) and (B) of the anthelmintic cyclodepsipeptide PF1022A (**143**) drew the conclusion[320] that biological activity is only maintained if the macrocyclic ring can preserve its symmetrical conformation. β-Turn mimetics, D-Pro–L-Pro and Nagai-Sato's BTD have been incorporated[321] into the backbone of (**143**) and only when these units were in the $i + 1$, $i + 2$ position was there a 2-fold increase in anthelmintic activity. Details of the first total synthesis[322] of apratoxin A (**144**) are testament to the researchers' abilities to overcome some critical 'sticky patches' along the route. For example, the thiazoline ring proved a very fragile moiety so had to be introduced at a very late stage *via* an azide as pro-amine group, which allowed an intramolecular Staudinger reduction/aza-Wittig to be performed. Macrolactonisation at the Pro carboxyl failed but macrolactamisation at position (a) succeeded. In the synthesis[323] of an analogue of (**144**) with an oxazoline instead of the thiazoline ring, the oxazoline ring could be assembled (using diethylaminosulfur trifluoride on a serine precursor) prior to macrocyclisation at point (a) using HATU/DIPEA. These cyclisation conditions were also chosen[324] to activate the Val-carboxyl in the syntheses of analogues, cyclo (Val–Trp–Ala–β-Ala–Ava), cyclo (Val–Trp–Ala–Ahx) and cyclo (Val–Trp–Ala–Aoc) of jaspamide, where Ava = 5-aminopentanoic acid, Ahx = 6-aminohexanoic acid and Aoc = 8-aminooctanoic acid. In the preparation[325] of the [*N*-(Mst)Ser[1], D-Ser[4], Thr[6], Asp[8], Thr[9]] syringotoxin analogue (**145**) the dehydro residue Dhb was incorporated as its Thr precursor followed by dehydration using EDCI, and the cyclisation at the Gly–D-Ser bond was effected using DIPCDI. α,α-Dialkyl substituted systems as summarised in Scheme 15 preferred[326] to dimerise to (**146**) than form the monomeric species.



Having personally experienced difficulties in the limited availability of chirally pure α-hydroxy acids, it was great news to read[327] about the successful development of the diazotisation reaction to encompass 19 of the amino acid analogues. The non-coded amino acid, 2-amino-3, 5-dimethylhex-4-enoic acid of cyclomarin A has also been synthesised.[328] New assignments of amino acid chiralities have had to be made to the structure of kahalalide F, so the native structure[329] now contains D-Val[3]–L-Val[4].



Scheme 15 Reagent: (i) HCl gas/toluene.

The binding of tamandarin A cyclo [Me_2Tyr–(Lac–Pro–D-MeLeu)Thr–Ist–Hiv–Leu–Pro] and the similarly structured didemnins to human elongation factor eEF1A has been subject to molecular dynamics simulation scrutiny.[330] The binding model presented involved, the 4-methoxyphenyl ring of the cyclodepsipeptides lodging into a hydrophobic pocket, formed by two protruding loops on the surface of eEF1A domain 2. The quantitative estimation of the histone deacetylase inhibitor FK228 was carried out by LC-MS,[331] beauverolides Q and R have been characterised[332] by tandem MS, the application of electron capture dissociation MS has been investigated[333] on valinomycin and beauvericin, and ion cyclotron resonance-FT-MS has been applied[334] to profiling members of the roseotoxins produced by *Trichothecium roseum*. Vibrational CD spectra of valinomycin and *ab initio* calculations have confirmed[335] its bracelet-type confromation. Molecular mechanics and dynamics calculations have been performed[336] on dolastatins 11 and 12 from the sea hare *Dolabella auricularia*.

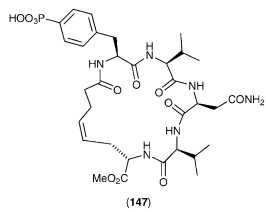
3 Modified and conjugated peptides

There continues to be expanding interest in this area which is mainly associated with post translational additions to the peptidic core of the molecules, and yet these represent key modifications that have fundamental consequences for molecular recognition. The interest in the field has coincided with key instrumental developments that have made possible analysis of some of the elusive structural features.

3.1 Phosphopeptides

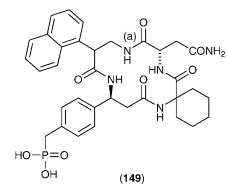
In a short review[337] the synthesis, purification and applications of phosphopeptides have been highlighted. Synthesis of phosphopeptides can offer two options, (i) addition of the phosphate post-amino acid assembly and (ii) using phosphorylated amino acids as building blocks. The former approach was the choice[338] to selectively phosphorylate Ser residues in tetra decapeptides related to the human insulin receptor using dibenzyl *N,N*-diethylphosphoramidate/1-H-tetrazole. Fragment peptides containing the acidic motif –Ser(P)–Ser(P)–Ser(P)–Glu–Glu from the casein have been synthesised[339] and on testing demonstrated that the influx of extracellular Ca^{2+} was due to the acid motif. Ring-closing metathesis[340] followed by phosphorylation [using bis (4-chlorobenzyl)-*N,N*-diisopropylphosphoramidite] were key stages in the formation of (**147**) and a hydrogenated analogue which could bind to the Grb2 SH2 domain. The phosphorylated analogue (**148**) of the lead compound G1TE which is known to bind to Grb2 SH2 domain turned out[341] to be an even more potent ligand (IC_{50} = 1.68 nM). The phosphorylated Tyr was introduced using Fmoc-Tyr[PO(OH,OBzl)]-OH. In the generation[342] of a phosphopeptide library on Tentagel beads, the phosphorylated Tyr-residue was introduced as Fmoc–Tyr-[$PO(OH)_2$]-OH, and the best sequence found for binding to STAT 1 was Tyr(P)–Asp(or Glu)–Pro(or Arg) and to STAT 3, Tyr(P)–Pro–Gln, where STAT means-signal transducer and activator of transcription. Several dipeptides and a tetrapeptide containing *O*-phosphoserine have been synthesised[343] using Ser(PO_3Ph_2) as the building block. When this unit was present at the C-terminus, it was more difficult to

activate the carboxyl group. A one-pot synthesis[344] of Fmoc–Ser[PO(OBzl)OH]–OH in high yield created the building block for the synthesis of the sequence, RKGS(PO_3H_2)SSNEPSSDSLSSPTLLAL related to the C-terminal of c-Fos protein. The use of caged phospho-amino acid building blocks has been extended[345] through the synthesis of new examples suitable for solid phase techniques. The caged compounds have photocleavable protecting groups as shown by Fmoc-phospho(1-nitrophenyl ethyl-2-cyanoethyl-serine, -threonine and -tyrosine, which were prepared using the reagent *O*-1-(2-nitrophenyl)ethyl *O′*-β-cyanoethyl, *N,N*-diisopropyl phosphoramidite. Conformationally-constrained phosphotyrosyl analogues useful for the study of cellular signal transduction have been reported[346] with examples ranging from Boc–(αMe)Phe(4-PO_3Et_2)–OH, Boc–Atc(6-PO_3Et_2)–OH to (±)(1*R*,2*R*,5*S*)3-acetyl-1,2,3,4,5,6,-hexahydro-8-*O*-phosphoryl-1,5-methano-3-benzazocine-2-carboxylic acid methyl ester. After synthesis,[347] (αMe)Phe(4-$CH_2PO_3H_2$), (αMe)Phe(4-$CF_2PO_3H_2$), (αMe)Phe(4-PO_3H_2) and both an S-acetyl thioester and a monobenzyl ester of phosphotyrosine have been incorporated as Xaa into inhibitors such as mAZ–Tyr(P)–Xaa–Asn–NH_2 of the Grb2 SH2 and found to provide affinity in the range 10^{-8} to 10^{-9} of K_d values, with the prodrug ester units showing potent antiproliferative activity. The Grb2 SH2 domains were also the targets[348] for macrocycles such as (**149**), which were constructed using FDPP at position (a). The central phosphoserine core of the PIN 1 substrate has been replaced[349] by *cis* and *trans* amide isosteres as in Ac–Phe–Phe–pSerΨ[*Z* and *E* (CH═C)]–Pro–Arg–NH_2. The *cis* isostere was 23-fold more potent than the *trans*.



(**147**)

(**148**)



(**149**)

N-Terminal derivatisation of peptides with ethoxy(phenyl)phosphinate, has given[350] phosphonamidate peptides useful for sequencing by mass spectrometry, while *N*-phosphoryl-Ser and -Thr, on ultrasonic irradiation[351] resulted in high yields of *O*-phosphoryl-Ser and -Thr.

Advanced NMR techniques[352] have been applied to the multi-phosphorylated motif contained within α(S2)-casein(2–20) in the presence of Ca^{2+}. A nascent helix conformation was deduced for residues $Ser(P)^9$-Glu^{12}, which seemed to differ from other casein phosphopeptides, such as β-casein(1–25) and α(S1)-casein(59–79). A continuum molecular electrostatics model[353] has successfully predicted the pK_a of the phosphate group in Gly–Gly–Tyr(P)–Ala. Binding free energies for the interaction of nine phosphotyrosyl peptides with the SH2 domain of Grb2 have been calculated[354] from molecular dynamics and surface plasmon resonance data, and quantitative structure-activity relationships have been investigated[355] using comparative molecular field analysis. ^{13}C and ^{31}P Chemical shielding tensors in solid *O*-phosphorylated amino acids have been worked out[356] from solid-state NMR spectroscopy and *ab initio* calculations.

Increased support for the newly labelled discipline of proteomics has triggered increasing interest in mass spectrometry techniques for the characterisation of phospho-proteins and -peptides. Some traditional chemical manipulations however prior to analysis can aid interpretation, and β-elimination[357] of the phosphorylated or acetylated groups of Ser/Thr followed by Michael addition of sulfite and ethanethiol would be typical. However conditions have to be kept mild to prevent dehydration of Ser/Thr centres which have not been derivatised. The influence of conditions on these eliminations has also been investigated[358] upon addition of propanedithiol and subsequent enrichment *via* solid-phase reversible binding.

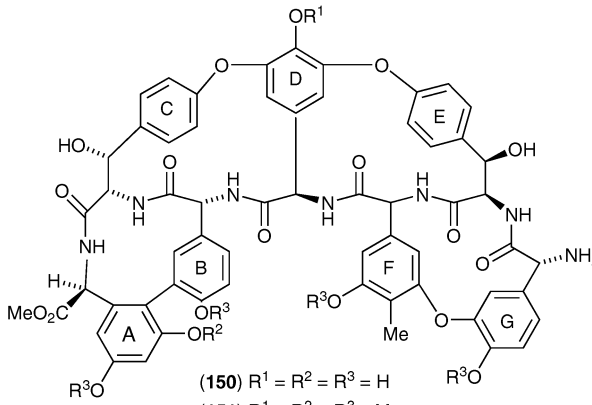
Hydrogen fluoride-pyridine or hydrofluoric acid have been shown to cleave the phosphate moiety on Ser, Thr and Tyr residues without side reactions.[359] N-Terminal phosphopeptide sequencing[360] using mass spectrometry has benefited from prior treatment with tritium-labelled 1-S(^{3}H) carboxy methyl-dithiothreitol, which can give a quantitative assessment of the number of phosphate groups present using hplc. α-Diazo functionalised solid phase resins have been used[361] to isolate phosphorylated peptides from non-phosphorylated species.

With this widening interest in phosphopeptide analyses, space limitations preclude an in-depth assessment, especially in the context of papers describing the analytical technology rather than the chemistry. The following Table summarises the many approaches using mass spectrometric techniques.

Source/type of phosphopeptides	Type of mass spectrometric technique	Refs.
Membrane protein bacteriorhodopsin	Liquid–liquid extraction-MALDI/TOF	362
Xenopus laevis His^6-aurora A	Immob. metal-affinity chromatography(IMAC) electrospray (ES) –Tandem	363
Insulin receptor substrate 1 fragment	LC/ES-API-CID-MS	364
Class 1 phosphoinositide-3-kinase βγ	Micro RP-Hplc –MS	365
Phosphotyrosine peptides	CID/Ion Trap MS	366
Yeast N_2 permease reactivator kinase	Isotope labelling + MALDI/TOF	367
Phosphorylated/ glycosylated peptides	AP-MALDI Ion Trap	368
In vitro cultured human lung cells	Isotope labelling/MS	369
Enzyme digests from phosphoproteins	Neutral loss activation (Pseudo MSn)	370
Phosphopeptides separated by LC	Quadrupole linear Ion Trap (QLT) MS	371
CGMP –dependent protein kinase	TiO_2 precolumning/LC-ESI-MS/MS	372
Phosphorylated tau peptides	MALDI/TOF	373
Peptides from proteolytic digests	H_3PO_4/2,5 DHB added before MALDI/MS	374
Sucrose-phosphate synthase isoenzyme	D_2(5) ethanethiol label before LC-MS	375
Na cationized phosphotyrosine peptides.	AP-MALDI/Ion trap	376
Epidermal growth factor receptor	Nano ESI high resolution MS/MS	377
P120catenin and glycoprotein fetuin	IMAC/β-elimination/Michael addition MALDI	378
Membrane phosphoproteins from (Arabidopsis)	Anion exchange prior to IMAC then MS/MS	379
Protein kinase C	LC ESI-FT-ICR-MS	380
Epidermal growth factor receptor	LC/MS/MS	381

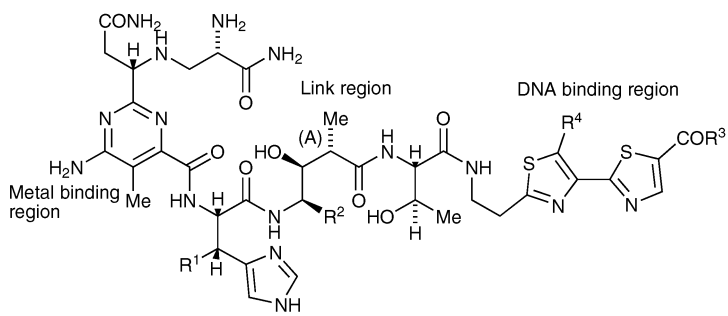
3.2 Glycopeptide antibiotics

This commercially important group continues to generate a great deal of interest, and some of the background work over recent years can be gleaned from reviews on the total synthesis of vancomycin,[382] nature's way of assembling vancomycin,[383] chemically modified vancomycins and their multivalent polymers,[384] the solid phase synthesis of the bleomycins[385] and the chemistry and biology of the ramoplanin family.[386]



(**150**) $R^1 = R^2 = R^3 = H$
(**151**) $R^1 = R^2 = R^3 = Me$

The 'pièce de résistance' in this section is the first total synthesis[387] of ristocetin aglycon (**150**). The approach benefited from the Boger group's previous synthesis of teicoplanin, which also has Cl atoms in ring C and E, and was based on the assembly of the left side of the molecule (rings ABCD) and the right side (rings EFG). Intramolecular aromatic nucleophilic substitution was instrumental in the closure of the C-D ring system, a Suzuki coupling reaction installed the A-B biaryl linkage followed by a macrolactamisation for closure of the 12-membered A-B ring system. A S_NAr reaction involving an *o*-fluoronitroaromatic ring created the F-G diaryl ether junction, which was followed by macrolactamisation steps and a coupling of the two main fragments before the final D-E ring closure.



bleomycin A_5 R^1 = carbohydrate, R^2 = Me, $R^3 = NH(CH_2)_3NH(CH_2)_4NH_2$, R^4 = H

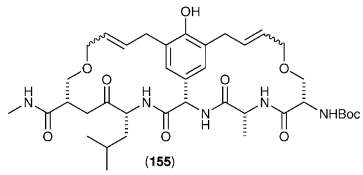
(**152**) R^1 = H, $R^2 = PhCH_2$, $R^3 = NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, R^4 = H

(**153**) R^1 = H, $R^2 = PhCH_2$, $R^3 = NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, R^4 = Cl

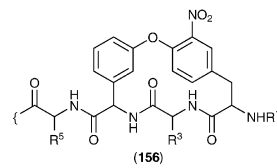
(**154**) deglycobleomycin A_5 with R^1 = OH

As part of the process to obtain a sample of the aglycone for comparison it was revealed[388] efficient cleavage of the sugar units in this series occurs with anhydrous HF at low temperature. Methyl ether derivatives of aglycon analogues of ristocetin *e.g.* (**151**), and of vancomycin and teicoplanin have been produced[389] *via* Boc-derivatised analogues treated with $TMSCHN_2$ in benzene/methanol. These

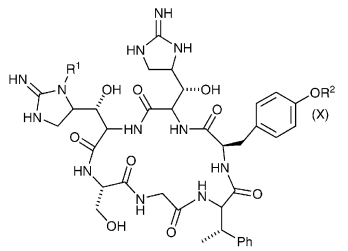
derivatives showed increased activity against VanB resistant strains of bacteria. The bleomycins which are able to mediate the sequence-selective oxidative damage of DNA and RNA, have been further investigated through the construction[390] of a 108-member deglycobleomycin library. Two analogues (**152**) and (**153**) mediated plasmid relaxation to a greater extent than the deglycobleomycin parent. The same solid phase protocols have enabled[391] conformationally-constrained analogues of deglycobleomycin A_5 (**154**) congeners to be prepared. Replacement of the methyl-valerate link unit (A) with a series of cyclic constraints did not aid DNA cleavage activity, but the analogues were competent in transferring oxygen to low M.W. substrates. Copper complexation provided the necessary protection to enable a series of analogues modified at the C-terminus, *i.e.* at the R^3 group in (**154**) to be prepared.[392] Test results showed that increased hydrophobicity at the C-terminus enhance antitumour activity. The vancomycin CDE ring motif has inspired the synthesis[393] of the bicyclic (**155**).



(**155**)

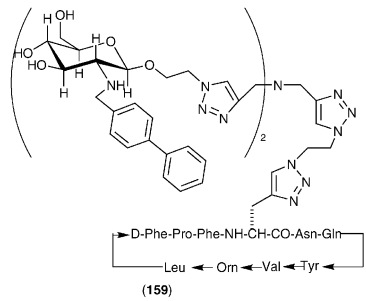


(**156**)



(**157**) mannopeptimycin β R^1 = sugar^1, R^2 = H

(**158**) mannopeptimycin α R^1 = sugar^1, R^2 = sugar^2



(**159**)

The central phenolic ring bearing two allylic groups was coupled to two allylated serine reidues using a second generation Grubbs catalyst to give (**155**), which appears to have a cavity-like based on modelling studies. Partial vancomycin mimics such as (**156**) have been prepared on-bead[394] using the ring closure afforded by a S_NAr displacement of a fluorine on the nitro-substituted ring. A number of lipophilic derivatives of various glycopeptide antibiotics, corresponding to changes at the sugar, resorcinol and amide moieties have shown activity[395] against HIV-1 and -2 and the Moloney murine sarcoma virus. A method[396] for attaching a peptide carrier domain (PCD) to the C-terminus of vancomycin aglycon involved PyBOP/thiophenol conversion of the C-terminus to a phenyl thioester, and reacting this with an apo-PCD under catalysis by phosphopantetheinyl transferase Sfp. Among the promising approaches to developing inhibitors of vancomycin-resistant enterococci (VRE) is the development of multivalent forms of the antibiotics. Thus a pyrene derivative[397] of vancomycin self-assembles due to π–π stacking in solution to form a dimer, and a multivalent vancomycin on the cell surface, while dimers of vancomycin linked by a rigid metal complex $[Pt(en)(H_2O)_2]^{2+}$ exhibited[398] potencies 720 times more than the parent antibiotic against VRE. Monomeric vancomycin synthons have been linked,[399] (by amphiphilic peptide-based linkers of different lengths) *via* their N- and C-terminii, the vancosamine residue and the resorcinol ring. *In vitro*-Antibacterial potency was affected by the orientation and the linker length (the shortest being the best), with links across the vancosamine residue providing the greatest

potency against VRE. Other links displayed more promising broad-spectrum activity. Amides produced from aliphatic, aromatic and heterocyclic amines at the C-terminii of vancomycin and LY264826 showed[400] excellent activity against staphylococci and streptococci. A number of benzoxazole derivatives assembled at point (X) in mannopeptimycin-β (157) have shown[401] good activity against Gram-positive bacteria, and a number of 6-*O*-ether and 4-*O*-ether derivatives of Sugar[1] in mannopeptimycin-α (158) exhibited[402] potent activity against VRE, MRSA and Gram-positive resistant strains. An efficient synthesis[403] of *N,O*-dibenzoyl-L-4-epi-vancosamine has appeared and a programmable one-pot oligosaccharide synthesis has been tried out[404] on vancomycin. A chemoenzymatic approach[405] featuring the thioesterase domain from tyrocidine synthetase, has shown that propargylglycine residues within the linear sequence can be tolerated, which can, after cyclisation be coupled with azido sugars *via* Cu (I)-catalysed cycloaddition to give glycopeptides such as (**159**) (therapeutic index 6-fold better than parent tyrocidine).

With antibiotic resistance a constant medical and political concern, developing an understanding of the binding interactions between antibiotics and the precursor peptidoglycan peptide terminus *N*-Acyl D-Ala–D-Ala (or *N*-Acyl D-Ala–D-Lac in VRE strains) has become paramount. Using Hartree-Fock and Density Functional Theory simulations on vancomycin and teicoplanin interactions, it has been calculated[406] that binding to Ac–D-Ala–D-Lac is weaker by 3–5 kcal/mol. By comparing the binding of Ac_2–Lys–D-Ala–D-Ala and Ac_2–Lys–D–Ala–D-Lac to vancomycin with that of Ac_2–Lys–D-Ala–Ψ[$COCH_2$]D-Ala, it has been found[407] that 2.6 kcal/mol worth of binding is lost because of lone pair repulsion between the vancomycin residue 4 CO group and the ester *O* in the Ala–Lac bond, compared to another 1.6 kcal/mol due to loss of H-bonding. A study[408] of the antibacterial properties of hydrophobic C-terminus amides of vancomycin, teicoplanin and eremomycin against Gram-positive strains and glycopeptide-resistant enterococci (GRE) showed better activity for the former rather than the latter. However by removing some of the glycopeptide-binding framework there was a decrease in anti-GRE activity, supporting the idea that two mechanisms of action are operating, a peptide core-interaction with the D-Ala–D-Ala cell-wall moiety and inhibition of bacterial membrane enzymatic reaction, which still operates in the GRE cases. A similar conclusion,[409] *i.e.* D-Ala–D-Lac binding is not required for high activity of vancomycin dimers against VRE, was based on the observation that tail to tail dimers of vancomycin and dimers of 'sequence damaged' vancomycins had similar high antibacterial activity against VRE. Configurational entropy and cooperativity between ligand binding and dimerisation have also been examined[410] using solvated molecular dynamics simulations and quasiharmonic normal-mode analysis on chloroeremomycin, vancomycin and dechlorovancomycin. It was concluded that cooperativity can be mediated through changes in vibrational activity irrespective of the presence or absence of structural change. Inconsistency in defining cooperativity has been clarified[411] through binding data revealed form NMR studies on the interaction between ristocetin A and cell wall peptide analogues. Interactions which are common in the binding of ligands are made with positive cooperativity with respect to those involved in dimerisation. Comparison[412] of known X-ray data on various glycopeptide antibiotics with dimerisation constants derived from NMR data showed that the latter can be correlated with tightness at the dimer surface, and account for the benefits in enthalpy and costs in entropy associated with positive cooperativity.

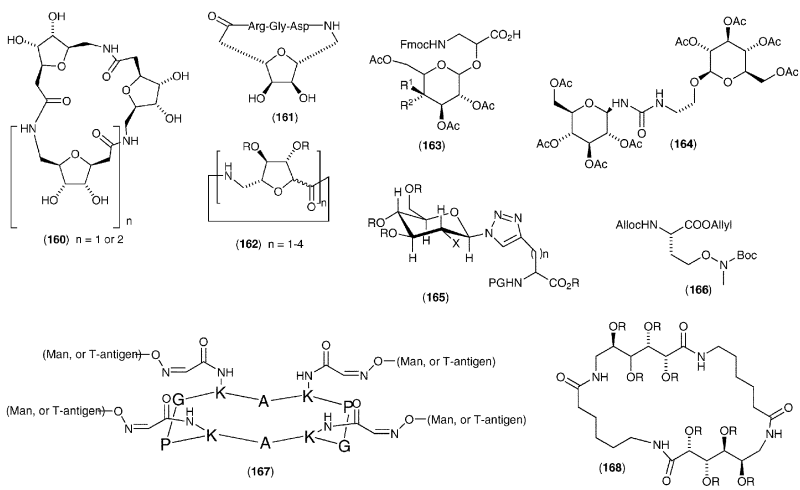
Structures have been worked out[413] for the four crystal forms of decaplanin. The structures contain the dimer units typical of vancomycin and family, but in addition there are a variety of further intermolecular interactions leading to intertwined 6_1-helices in two of the crystal forms. Vancomycin *in vacuo* is the intriguing title of a review[414] that brings together data from a decade of mass spectrometric studies on non-covalent interactions with ligands, and recent gas-phase studies on non-covalent complexes of vancomycin. Enantio-resolution using the vancomycin family

members as bonded chiral phases has been further explored with examples of phenyl isothiocyanated amino acids[415] being resolved, vancomycin analog A82846B proving to be better than vancomycin in capillary electrophoresis and HPLC,[416] hydroxy acid derivatives being resolved by capillary electrochromatography using a teicoplanin analog,[417] and in a mechanistic study[418] on the capillary electrophoresis separation of dansylated amino acids, the amino sugar moiety of the antibiotics and dimer formation are offered as explanations for the resolution.

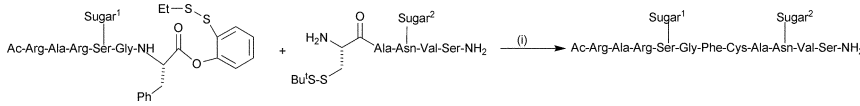
3.3 Glycopeptides

This sub-division has seen continuous expansion over the last decade, and as this coverage reports on two years of publications, this has to be a focussed account of the main achievements which involve the sugar peptide link. References are clustered together in terms of their content being predominantly *O*-, *N*-, *C*- or *S*-glycopeptide linkages, but some papers take a more general theme, while others do not fall naturally within a linkage. They are therefore discussed first.

Strategies for synthesising glycopeptides,[419–421] and chemoselective ligation[422] for assembly of neoglycopeptides have been reviewed. A microreview[423] concentrating on coupling glycopeptides to glycosylphosphatidylinositols has appeared, and both *O*- and *N*-linked glycosyl amino acid synthesis have been highlighted.[424] The derivatisation of the vancosamine sugar and the resorcinol ring of vancomycin to form the clinical antibacterial candidate TD-6424, has been reviewed.[425] Furanoid and pyranoid ε-sugar amino acids are proving to be useful for constructing interesting scaffolds, *e.g.* (**160**) and pyranoid equivalents, which can be made[426] from linear precursors assembled, cyclised and cleaved using an oxime (Kaiser) resin. Conformational analysis of (**160** $n = 1$) showed that the furanoid rings flip between a twist and an envelope, with the side chains connecting the CO functionality being more rigid than the ones on the other side of the rings. In a similarly synthesised[427] series of cyclic RGD analogues, compound (**161**) showed the most promising inhibition with IC_{50} values of 1.49 μM for the $\alpha_v\beta_3$ and 384 nM for the $\alpha_{IIb}\beta_3$ receptors. The BOP reagent was instrumental[428] in the macrocyclisation yielding the homooligomers (**162**, variable stereochemistry at the carboxy terminus), which were found to have symmetrical structures in solution.



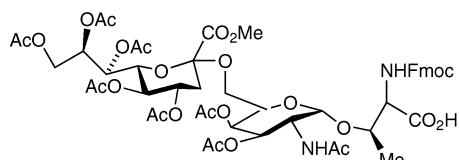
The synthetic road to the larger glycoproteins has been made easier by the development[429] of a method for ligating both *O*-and *N*-linked glycopeptides together as summarised in Scheme 16. *O*-Glycosylated- (**163**), *N*-glycosylated and *O*-, *N*-glycosylated- (**164**) derivatives of isoserine represent[430] new glycosylated β-alanine



Scheme 16 Reagents: excess MES-Na pH 7.4.

surrogates. New insights[431] into the Maillard reaction focuses on the physico-chemical properties of the Amadori compounds of bioactive peptides such as enkephalin and morphiceptin, while glycosyl phenyl thiosulfonates[432] allow rapid glycosylation of peptides and proteins. Dendritic sialyloligosaccharide-displays attached to an N-terminal Gln at the B1 position of insulin[433] cause a prolonged blood sugar lowering activity. First examples of triazole-linked units such as (**165**) have appeared,[434] and a versatile set of aminoxy amino acids [*e.g.* homoserine derivative (**166**)] have been synthesised[435] for access into neoglycopeptides. An aminoxy function was also the linker of choice between a cyclic decapeptide template[436] and four mannosyl or T-antigen units as summarised in (**167**). Hydrogenation of linear ω-azido-pentafluorophenyl esters and ε-amino acids have yielded[437] 14-, 21-, 28-, 35-, 42-, 56- and 70-membered cyclic lactams of the type represented by the 28-membered (**168**).

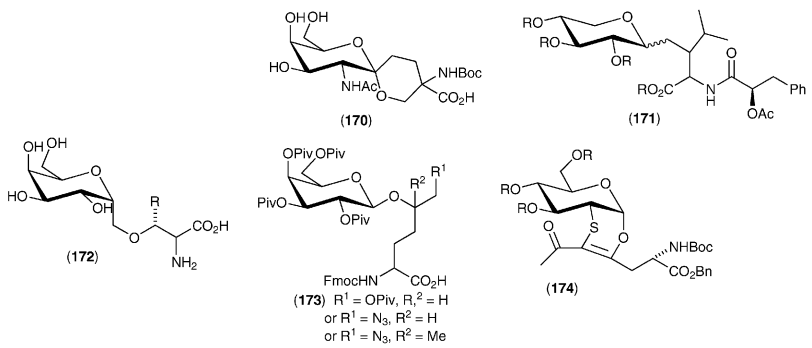
3.3.1 O-linked glycopeptides. The first synthesis[438] of the 'polar mycoside C' glycopeptidolipid has appeared, based on the disconnection of the structure, 3,4-di–OMe–α–L–Rhap-(1 → 1)}*R*-$C_{21}H_{43}CH(OH)CH_2CO$–D-Phe–[4-OMe-α–L-Rhap-(1 → 4)-2-OMe-α-Fucp-(1 → 3-α-L-Rhap-(1 → 2)-6-deoxy-α-L-Talp–(1 → 3)]–D-aThr–D-Ala–Alaol} into four building blocks, with the *O*-link to aThr created *via* a pentenyl glycoside. Glycosyl bromides were used[439] to produce Fmoc-protected Ser building blocks with the Ser attached separately to β-D-Glc, α-D-Glc(1 → 4)-β-D-Glc and [α-D-Glc(1 → 4)]$_4$-β–D–Glc. These were used for the assembly of the enkephalin, Tyr–D-Thr–Phe–Leu–Ser(R)–NH_2 glyco-derivatives on a Rink amide resin. NMR, CD and molecular mechanics showed that the glycolysis did not perturb much of the peptide backbone in aqueous solution, but underwent profound membrane-induced conformational changes. Glycopeptides have been synthesised which contain tumour-associated saccharide antigens, such as the Thomsen-Friedenreich (T), T_N or sialyl T_N in combination with peptides from the tandem repeat region of MUC 1. Much of the recent work on synthesis has been gathered together in a review.[440] The same research group have reported on synthesis of glycopeptides for development of antitumour vaccines,[441] as partial structures of the homophilic recognition domain of epithelial cadherin,[442] the repeat sequences[443] of epithelial MUC 1 and MUC 4. Selective thioester activation has coupled together[444] two segments bearing core 2 sialosaccharides as mimics of the heterogeneous surface of mucin glycoprotein, while 2-nitrogalactal concatenation was a key step[445] in forming building blocks such as (**169**) employed in the synthesis of a mucin repeating sequence. Fmoc Ser (or Tyr) [β-D-Glc(OAc)$_4$]–OPfp building blocks formed from the Fmoc-active esters reacting with β-D-Glc(OAc)$_4$ in the presence of $BF_3 \cdot Et_2O$ did not need purification before incorporating into glycopeptides by solid phase synthesis.[446]



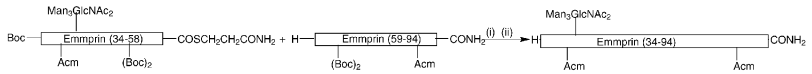
(**169**)

Chemo enzymatic techniques[447] have secured GM3 oligosaccharides on to the Ser side-chains in cyclo (Ser–Gly–Gly–Gln–Ser–His–Asp)$_3$. Inhibition effects on

hemagglutination, by the influenza virus was greater with three ligands, and was sensitive to the sequence used in the cyclic peptide. Fmoc-homoSer(α-D-GalNAc)–OH has been incorporated[448] as trimeric clusters into the vaccine [MAG:Tn(hSer)$_3$-PV] which showed a strong antibody response, and magic angle spinning NMR techniques have monitored[449] the direct *O*-glycosylation of residues bound to Tentagel resin using glycosyl trichloroacetimidate donors. Mimics related to anti-freeze glycoproteins have been more efficiently synthesised[450] using 1-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ) or 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) as polycondensation agents than with using DPPA. Practical synthesis[451] of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy β-D-glucosides of Fmoc-Ser and Fmoc Thr and their benzyl esters has been reported, and Fmoc-β-D-Glc–(*S*)-α-MeSer–OBn has been chosen[452] as a conformationally-constrained building block. The constrained unit (**170**) has also been obtained[453] but to obtain pure stereochemical forms, enzyme-catalysed de-symmetrization of an intermediate had to be carried out. α- and β-Forms of the N-terminus moiety (**171**) of aeruginosin 205-A have been synthesised,[454] and both α-*O* and β-*O*-linked glycosyl amino acids were obtained using monosaccharide tri-chloroacetimidate donors (Tn, TF, STn, Lewis(y) or Globo-H)with Fmoc-hydroxynorleucine benzyl ester.[455] The biological indicator of collagen turnover, Glu–Gal–Hyl, has been synthesised,[456] and galactosylated 5-hydroxylysine building blocks such as (**172**) have been prepared.[457] It has been possible to create[458] *O*-glycosyl mimetics incorporating an extra methylene group in the link as shown in (**173**). These proved to be stable in the presence of glycosidases and showed competitive inhibiton of α-galactosidase. Glycals have been shown[459] to add stereochemically to Asp derivatives to yield α-*O*-linked glycohomoglutamates such as (**174**), while α-D-mannosylphosphate serine derivatives have been synthesised[460] by reaction of α-mannosyl phosphoramidites with protected serine in the presence of 1H-tetrazole.

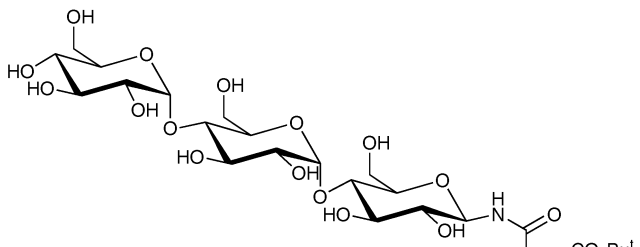


3.3.2 N-linked glycopeptides. The total synthesis[461] of hybrid-type gp120 fragments, in pursuit of carbohydrate-based HIV vaccines, has relied heavily on the Kotechkov amination of the sugar unit which can then be coupled to the carboxyl side-chain of aspartyl residues. Prospects for a totally synthetic vaccine targeting epithelial tumours have been surveyed[462] by concentrating on developing a pentameric vaccine containing prostate tumour associated antigens Tn, TF, STn, Lewis(y) and Globo-H. The antigen-containing amino acid monomers were assembled in a linear fashion to form a glycopeptide containing five distinct carbohydrate antigen units. Native chemical ligation[463] between H–Cys (SBu^t)–Ala–Asn[(Manα1)$_2$-3,6Glcβ1 → 4GlcNAcβ1 → 4GlcNAcβ1]–Ala–Ser–NH_2 and H–Gly–Asp–Ser–Ala–Trp–His–Leu–Gly–Glu–Leu–Val–Trp–Ser–Thr–Gly–S$(CH_2)_2$CO–NH_2 in the presence of $HSCH_2CH_2SO_3Na$ afforded the 20-mer glycopeptide after hplc purification. A similar ligation[464] summarised in Scheme 17 followed by further processing of the Acm-protected Cys residues to give the disulfide ring gave the required first Ig



Scheme 17 Reagents: (i) AgCl, HOOBt (ii) TFA.

domain of the glycoprotein, emmprin, which occurs on the surface of cancer cells. Both solid and solution phase protocols[465] have been reported for the CD 52 glycopeptide, Ac–Gln-Asn(β-D-GlcNAc(1 → 4)[α-L-Fuc(1 → 6)]-β-D-GlcNAc)–Asp–Thr–Ser–Gln–Thr–Ser–Ser–Pro–Ser–OH. The fucosylated trisaccharide was linked to Asn to make the building block, and in the protocol, DCC/HOBt proved to be the best coupling agent. Fmoc-Asn bearing unprotected trisaccharide chains (**175**) afforded[466] a means of coupling units in homogeneous solution in *N*-methyl pyrrolidinone, with ether being used to precipitate the peptides. Because of H-bonding between the free oligosaccharides high efficiency was achieved in the couplings of two glycosylated building blocks. By using an Asn-linked sialyl glyco-amino acid from egg yolk, the free carboxyl groups in the sialyl unit can be converted[467] to benzyl esters which make the unit tolerable to 95%TFA, and hence can survive the conditions of solid phase synthesis.

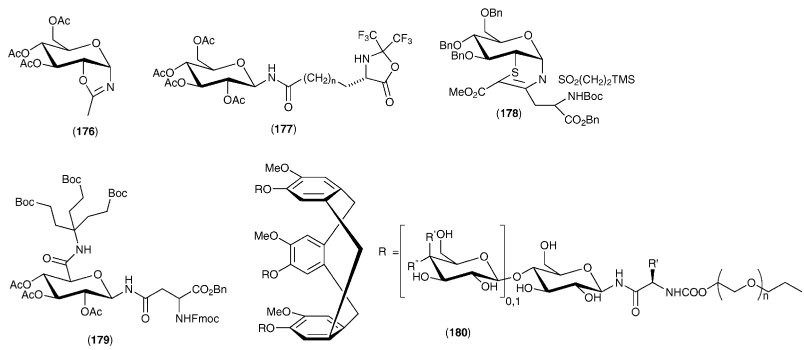


(**175**)

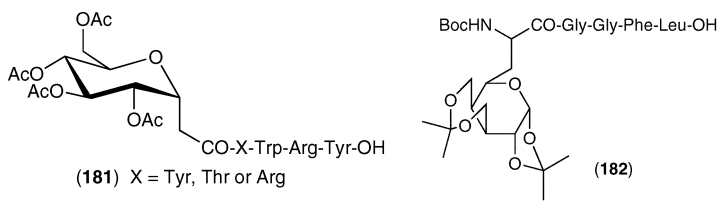
Chemoenzymatic approaches have an honourable track record in this field, and in this review period are exemplified by the egg yolk extract discussed above being modified[468] by Fmoc and benzyl derivatisation, have been subjected to branch-specific exo-glycosidase digestion (α-D-neuraminidase, β-D-galactosidase, N-acetyl-β-D-glucosaminidase and α-D-mannosidase). The Asn-linked compound thus obtained could be used in solid phase synthesis using acid-labile HMPA-PEGA resin. Recombinant Arthrobacter Endo-β-N-acetyl-glycosaminidase has catalysed[469] transglycosylation to acetylglucosaminyl peptide to give high-mannose type HIV-1 gp 120 glycopeptides. The oligosaccharide moiety in a synthetic study of glycopeptides has been provided[470] by the use of endo-β-GlcNAc-ase on a GlcNAc-peptide.

An *N*-glycosyl-Asn link has been created[471] *via* the photochemical coupling of a photoreactive amide of an Asp side chain with an aminosugar, and novel conjugates have been produced[472] through the coupling of amino acids with aldehyde functionalised carbohydrate derivatives and reductive amination. Microwave-assisted Kochetkov aminations[473] at 250 psi have given aminosugars which can be coupled to the Asp side-chain using HBTU/HOBt. Isoxazolines such as (**176**) when reacted with Z-Asp(S-pyridyl)-OBn in the presence of $CuCl_2$ gave exclusively[474] α-N-glucopyranosyl-Asn derivatives, while Me_3SiN_3/Me_3SiONO_2 converted[475] glycals to 1-azido 2-deoxy sugars, which after reduction of the azide to an amino group provided functionality to couple with amino acids. *N*-Glycoside building blocks such as (**177**) have been formed[476] by reaction of hexafluoroacetone-protected ω-activated Asp, Glu derivatives and glycosyl amines, while (**178**) represents a novel neoglycopeptide building block.[477] Building block[478] (**179**) allows further branching of the carbohydrate unit to form dendronised saccharides, while the scaffold (**180**) can be used[479] to study multivalent interactions. A bromoacetamidyl disialyl

undecasaccharide has been coupled to the Cys side chain of H–Arg–Glu–Glu–Gln–Tyr–Cys–Ser–Thr–Tyr–Arg–Val–OH and the resulting glycopeptide was stable to enzymatic digestion.[480]

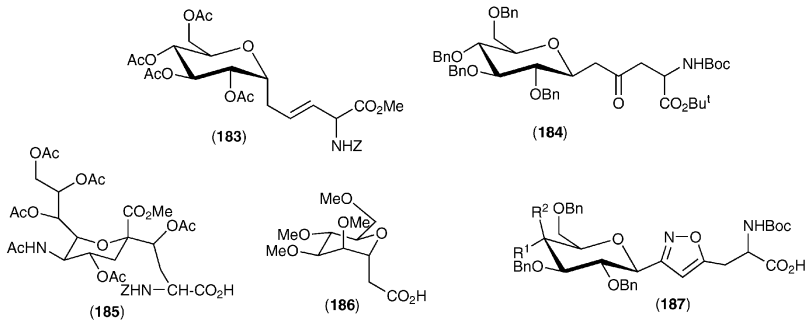


3.3.3 C-linked glycopeptides. The added stability of C-glycosidic links has made this combination a favourite mimetic. Yet nature has come up with its own C-linked analogues[481] which initiated the synthesis of C^2-α-D-C-mannosylpyranosyl Trp from benzyl protected 1,2-anhydromannose and a lithiated indole derivative. C-Linked analogues of antifreeze glycoprotein have been prepared[482] and although 'weaker' than the native protein in activity they did bind to ice. In a C-linked glycopeptide library,[483] examples represented by (**181**) offered the best inhibitors of the binding of human natural anti-Gal antibodies. The protected Leu enkephalin analogue (**182**) represents the penultimate stage to the free galactosyl peptide, and shows the form of the glucidic amino acid which can be incorporated into peptides.[484]

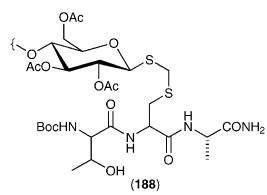


The synthesis of *C*-glycoside analogues in a suitable form to function as building blocks for solid phase synthesis has been popular. Fourteen synthetic steps[485] were required to make a *C*-glycoside analogue of β-D-galactosylthreonine, while the alkene bond in (**183**) represents the position reached[486] (prior to hydrogenation) on coupling α- or β-*C*-allyl glycosides with vinyl glycines using Grubbs's catalyst, to afford *C*-glycosyl Asn's. Stereocontrolled addition[487] of functionalised allylsilane to activated sugar derivatives, followed by an alkylation of a glycine imine, gave (**184**), while compatibility of protecting groups with samarium diiodide became a requirement in the synthesis[488] of neuraminic acid analogue (**185**). *C*-Glycosyl amino acids have been produced[489] from reaction of 1-methylenesugars with nitrones to give spiroketosyl isoxazolidines which ring opened with Zn/HOAc, and a Pd-catalysed Stille Neghishi cross-coupling reaction secured the formation[490] of *C*-glycosylated Phe. *C*-Sugar derivative (**186**) was shown to greatly alter the water solubility of leucine when it was linked to the amino acid,[491] and *C*-glycosides, *p*-methoxybenzylamine and a ketene silyl acetal mixed in a one-pot reaction catalysed by $InCl_3$ have yielded[492] *C*-galactosyl and *C*-ribosyl β-amino acids. 1,3-Dipolar cycloadditions of

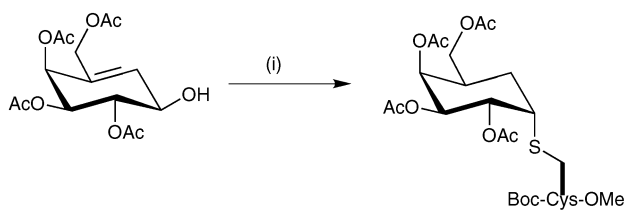
C-glycosyl nitrile oxides to either an alkyne or an azide have produced[493] either the isoxazole (**187**) or its triazole equivalent.



3.3.3 S-linked glycopeptides. A modest increase in publications on this type of linkage justifies a sub-group listing of their own. Two approaches to the synthesis have been carried out successfully in aqueous solution. One approach[494] used the chemoselective ligation of bromopeptides with 1-thiosugars, while the other approach[495] involved ligation of bromo sugars, *e.g.* glucosyl bromide, galactosyl bromide or *N*-Troc protected 2-amino-2-deoxyglucosyl bromide, with Cys or hCys residues in peptides, in the presence of sodium carbonate. Glycosyl thiomethyl azides with Asp or Glu thioacids have given[496] glycosyl thiomethyl amides, and glycosylthiomethyl bromide with Cys residues gave (**188**). 1,4-Conjugate addition[497] of oligosaccharide thiolates (of antigens T_N, T, ST_N and 2,6-ST) to dehydroalanine containing peptides gave good yields of *S*-linked mucin-related conjugates with total retention of α-anomeric configuration. Reactions of 1-thiosugars with Cys-rich oligopeptides under slightly basic conditions under gentle air oxidation created[498] a library of disulfide linked conjugates, with members being active enough to cross-link peanut lectin molecules. Scheme 18 summarises the last stage in forming a sugar-Cys link after a Grubbs' catalyst induced[499] formation of the unsaturated sugar analogue. α-Thiolated sugars were amongst nucleophiles used[500] to react with aziridine-2-carboxylic acid residues within peptides to yield *S*-sugar links such as Ac–Lys–Ser–Glu–Gly–Phe–Cys(GalNAcα-1)Ala–OH.



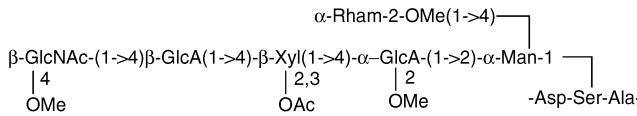
All types of conjugate links reviewed here have benefited from the thrust in development of mass spectrometric techniques created by the proteomics era. Applications of MALDI mass spectrometry to this area have been reviewed[501] as



Scheme 18 (i) MsOH, Boc-Cyc-OMe.

are the recent advances in the glycopeptide analysis.[502] Selective detection can be carried out by ion trap mass spectrometers,[503] while multistage MS with low-energy collision-induced dissociation offers complete structure elucidation.[504] Glycosylation analysis of immunoglobulin G was carried out[505] using capillary LC-Q-TOF MS, and localisation of *O*-glycan links using MALDI techniques has benefited[506] from conversion of glycopeptides to acetyl phosphonium derivatives. MALDI-FT MS has been used[507] to locate *N*-glycosylation sites, and a comparison[508] of the *N*-glycosylation status of human lactoferrin from maize and tobacco plants has been carried out. The fragmentation of the *N*-glycosylated Asn link in MALDI/TOF MS has been studied.[509]

Conformations around the *O*-glycosidic link in [GlcNAc-β(1 → 4)-GlcNAc-α]-Asn [286] hemagglutinin(181–190) peptide have been explained after detailed analysis of couplings,[510] and NMR analysis[511] of adamantyl or Boc-Tyr derivatives (linked *via* the Dap residue) of peptidoglycan monomer from the cell wall of *Brevibacterium divaricatum* revealed conformational changes but without loss of activity. NMR, X-ray crystallography and molecular dynamic simulations[512] on cyclic furanoid ε-sugar amino acids within a cyclic tetramer, and containing RGD residues showed that the furanoid residues introduce an unusual H-bond stabilised β-turn-like conformation in two out of the three cyclopeptides studied. The fish pathogen *Flavobacterium columnare* released the minimal common tripeptide (**189**) on proteolytic digestion.[513] Further evidence has been accumulated[514] from both experimental and computational data that the conformations of peptides and α-and β-linked Asn glycopeptides are uniquely influenced by the attached saccharide. To overcome the loss of glycosylated links during Edman degradations, in single bead analysis of cyclopeptide templates, a cleavable sugar-peptide linker has been employed.[515]

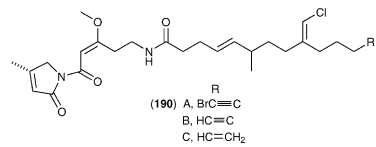


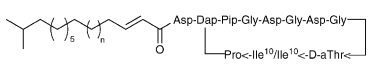
(**189**)

3.4 Lipopeptides

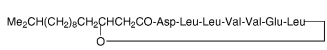
An extensive screening programme on the marine cyanobacterium *Lyngbya majuscula* led to the isolation of jamaicamides A–C (**190**),[516] and all possible *C*-4 and *C*-5 diastereoisomers of antillatoxin from the same source have been bioassayed,[517] and in all assays the natural (4*R*,5*R*)-isomer was greater than 25-fold more potent than the rest. The natural isomer was also shown to have an 'L-shaped' topology with an accumulation of polar substituents on the external surface of the macrocycle. An echinocandin class member, A-192411 (**191**) has shown[518] novel fungicidal features and has been synthesised from a linear precursor by cyclisation (60% yield) at position (a) in (**191**) using DPPA/$NaHCO_3$. Glycinocins A to D (**192**) have been identified[519] in an unidentified Actinomycete species, while the thermophilic subsurface *Bacillus licheniformis* strain 603 generated[520] the antiadhesin 1 (**193**) which is an inhibitor of bacterial adhesion. *Pseudomonas putida* strain PCL 1445 has yielded[521] two biosurfactant cyclic depsipeptides, putisolvin I and II, as in (**194**), while *Pseudomonas fluorescens* yielded[522] two novel antibiotics Sch 419558 (**195**) and Sch 419559 (**196**). One of two new cytotoxins isolated from cyanobacterium *Symploca* has been identified[523] as (*R*)-3-methoxyhexanoyl–Phe–Me–D-Val–Val–MeIle–MePhe–MeGly–Thz. Out of a series of four palmitoylated peptides which contained positively charged residues and D-forms (italicised), only Palm-*K*GGG*K*WGG*K*GG*K*-NH_2 and Palm-KA*AA*KWA*A*K*A*AK-NH_2 gained potent antibacterial and antifungal activity despite both parental peptides being devoid of activity.[524] Kalkitoxin (**197**) from cyanobacterium *Lyngbya majuscula*, has been

synthesised[525] in 16 steps (3% yield) using asymmetric organocopper conjugate addition and an *in situ* enolate alkylation. Semisynthetic modification[526] of the echinocandin, mulundocandin, at the Orn[5] residue, showed that the majority of analogues retained the same activity with improved aqueous stability, but the thiophenol analogue had improved antifungal activity when fed to Swiss mice. Constructs at both the *N*- and *C*-terminus of alamethicin F30 sequence have been designed[527] to exhibit a prolonged lifetime and higher membrane activity.

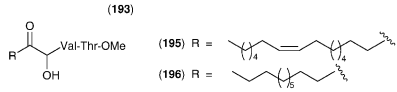


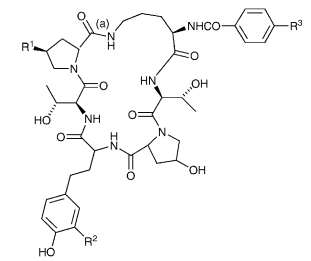


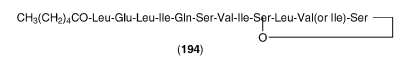
(192) A, n = 1; B, n = 2; C, n = 0; D, n = 1,Val[10]



(193)



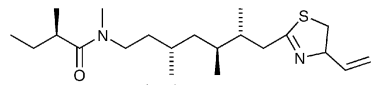




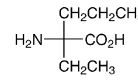
(194)

Fullerene conjugates such as (**198**), or tripalmitoyl-*S*-glyceryl-Cys derivatives at the *C*-terminus resulted in pronounced ion channel stabilisation, in contrast to the disturbance of the channels with *N*-terminal conjugates. Solution phase protocols[528] have been used to synthesise the lipopeptaibol metabolite LP237-F8, Oc–Aib–Pro–Phe–Aib–$(Gln)_2$ –Aib–Etn[8]–Gln–Ala–leucinol, from the fungus *Tolypocladium geodes*. The Etn residue (**199**) had to be asymmetrically synthesised, and when replaced by Aib or Nva at position 8 all three analogues were highly folded in solution and caused membrane lysis. Production[529] of a library of side-chain ornithinyl derivatives (formed through aldehyde condensation and reduction) of the lipocyclodepsipeptide, daptomycin, showed that sulfonamide, amide or polar spacers such as piperazine beneficially affected activity. Full experimental data have been reported[530] for the solid phase synthesis of farnesylated and palmitoylated lipopeptides using a hydrazide linker cleaved by oxidation with copper acetate. Although the same linker could be used, above the decapeptide level a strategy using pre-lipidated Fmoc-protected building blocks was more successful.[531] Lipidic side chains can also be added[532] on-resin to an assembled peptide which has aldehyde side-chains camouflaged as *N*-methoxy, *N*-methyl amides. Four copies of a cyclic disulfide epitope (a version of the antigenic site A of FMDV virus) has been conjugated[533] *via* a peptide bond to a lipidated branched Lys scaffold. The immunogen was successfully used in a vaccination trial against foot and mouth disease. The ester bonded side chains of *N*-palmitoyl-*S*-[2,3-bis(palmityloxy)-propyl–Cys–Ser–$(Lys)_4$–OH have been shown[534] to account for its powerful adjuvanticity to HLA class 1-restricted CD8(+) T lymphocytes, and an oligopeptide, $Me(CH_2)_{16}CO$–Gly–Ala–Asn–Pro–Asn–Ala–Ala–Gly–NH–$(CH_2)_{16}Me$ modified with stearyl moieties on both *N*- and *C*-terminii and anchored to a liposome was forced into a β-hairpin conformation which would otherwise be random coil.[535] The solution structure of the cytolytic lipodepsipeptide tolaasii from *Pseudomonas tolaasii* in sodium dodecyl sulfate studied[536] by 2D-NMR and molecular dynamics, assumed an amphipathic left-handed α-helix in the region D-Pro^2-D-$aThr^{14}$ believed to be the first example including both D- and L-amino acids. A high field NMR study[537] of lipopeptide daptomycin (used clinically for skin infections as Cubicin)

and its binding to calcium ions, has shown that the calcium ions do not result in major conformational changes but do induce aggregation. For the cyclic lipopeptides, iturin and surfactin, complexes with silver, nickel and strontium ions enhanced[31] abundances of fragment ions in MS, while MALDI-MS of Bacillus isolates from sponge *Aplysina aerophoba* showed[538] lipopeptides such as, surfactins, iturins and fengycins to be the bioactive metabolites. The MALDI-MS protocol has also found application[539] in the detection of fengycin and surfactin from *Bacillus globigii*, and using a modelling analysis[540] the C15 homologue of surfactin appears to be the most active with membranes.



(**197**)



(**199**)

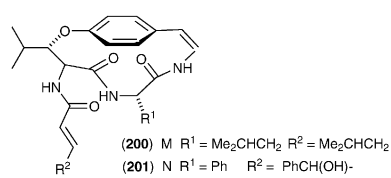
Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phe —$NH(CH_2)_2NHCONH$-C_{60}/C_{70}

(**198**)

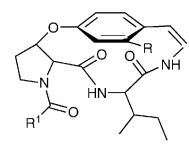
4 Miscellaneous structures

Interesting papers that somehow cannot be directed into other sub-sections of this Chapter, have traditionally been discussed here. Cyclic peptide alkaloids have annually being covered here, this time represented by discarine M (**200**) and N (**201**) identified[541] amongst other known peptide alkaloids in the bark of *Discaria Americana*, and ramasonines A–C (**202**), hemsines, A/B (**203**), C/D (**204**) from the roots of *Paliurus ramosissimus* and *P. hemsleyanus*, respectively.[542] The psychrotolerant fungus *Penicillium rivulum* has afforded[543] two new cyclic nitropeptides psychrophilins B (**205**) and C (**206**). The macrocycle of C3-epimauritine D (**202** with R=H, R′=Me_2Ile-Leu) has been constructed,[544] macrocyclisation at the Pro–Ile bond being carried out using tetramethylfluoroformamidium hexafluorophosphate (TFFH)/HOAt.

Lanthionine-type analogues[23] of urotensin II with structures H-cyclo[Xaa–Phe–Trp–Lys–Tyr–Cys]–Val–OH where Xaa = Abu or Ape (aminopentanoic acid) had their sulfide link incorporated on resin using (2S)-Fm-2(Boc-amino)-4-iodobutanoate or its homologue 5-iodopentanoate using microwave irradiation, followed by macrocyclisation on-resin at Xaa-Phe using HBTU/DIEA.

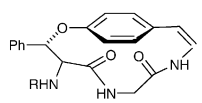


(**200**) M $R^1 = Me_2CHCH_2$ $R^2 = Me_2CHCH_2$
(**201**) N R^1 = Ph R^2 = PhCH(OH)-

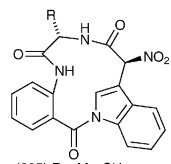


(**202**) A, R = H, $R^1 = Me_2$Ile
B, R = H, R^1 =MeIle-Phe-
C, R = OH, $R^1 = Me_2$Phe

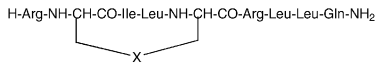
(**203**) A, R = H, $R^1 = Me_2$Trp
B, R = H, $R^1 = Me_2$Ile-Phe-



(**204**) C, R = Me_2Trp-Pro-
D, R = MeVal



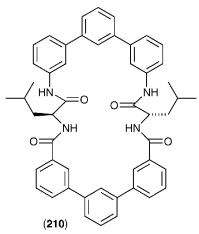
(**205**) R = Me_2CH
(**206**) R = Me

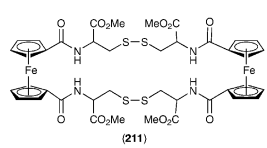


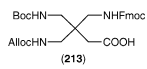
(**207**) X = -S-S- (**208**) X = -S- (**209**) X = -S-CH_2-

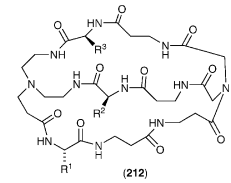
Nickel boride in the presence of deuterium gas to reduce dehydro side chains and desulfurise lanthionine bridges, has successfully located[545] the dehydro and

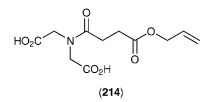
lanthionine bridge sites in lacticins 3147 A1 and A2 confirming similiarities with type B lantibiotic, mersacidin, and the type A class, respectively. The signature nuclear receptor box motif has been incorporated[546] into analogues (**207–209**), and of the three, the cystathionine (**207**) showed up best in inhibition studies on estrogen receptor-coactivator. Macrocyclic scaffolds capable of functioning as anion receptors have been the subject of review.[547] A variety of functionalised 3,3′,5,5′-tetraaryl-1,1′-biphenyls such as (**210**) have been synthesised[548] and shown to be capable of forming 'nesting' complexes with octylglucosides. Of a family of ten ferrocenyl-bearing cyclopseudopeptides,[549] the compound (**211**) had K_1 binding constant of 4.37×10^6 mol^{-1} L and a Ca^{2+}/K^+ selectivity of 300 000:1 making it better than valinomycin. The cryptand-like molecule (**212**) has benefited from being synthesised[550] on solid support, and the branching units (**213**) and (**214**) used on a hydroxymethyl–functionalised polystyrene support were key to the solid-supported synthesis of bicyclic peptides.[551]


(**210**)

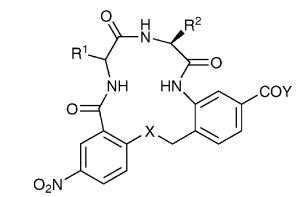

(**211**)


(**213**)


(**212**)


(**214**)

It has been confirmed experimentally[552] that only if cyclo[(Gln–D–Tle–Glu–D–Tle)$_2$] is present in the presence of its enantiomeric equivalent, cyclo [(D-Glu–Tle–D-Glu–Tle)$_2$ will the sterically hindered t-leucyl (Tle) residue, allow nanotube formation. Computer modelling and Raman/IR microspectroscopy have shown[553] that cyclo $(NHCH_2CH{=}CHCH_2CO)_3$ dissolved in nematic liquid crystals form large hexagonal hollow tubes. A second generation of 14-membered ring β-turn mimics (**215**) have been synthesised[554] by solid phase with the final macrocyclisation involving displacement of fluoride from the nitrated aromatic ring. The analogue with an amino bridge showed the least tendency to form a β-turn.


(**215**) X = O, Y = NH_2, or X = S, Y = OH, or X = NH, Y = NH_2

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Metal complexes of amino acids and peptides

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1. Introduction

This chapter covers the most important results and observations published on the synthesis, structure, kinetics and reactivity, solution equilibria and various applications of the metal complexes of amino acids, peptides and related ligands in the years 2003 and 2004. The major source of the references reported here is CA Selects on Amino Acid, 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 hard copies of the above-mentioned journals, the Abstracts reported by the Web of Science Databases on the Internet[2] have also been searched.

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 types of ligands. In addition to the naturally occuring amino acids and peptides, results for some synthetic analogues were also considered if they had biological or theoretical significance or important applications in chemistry, medicine or industry. The traditional structural, kinetic and thermodynamic characterization of the complex formation processes of these ligands is still a matter of interest, with the number of publications having increased especially in the fields of analytical and biomedical applications of the complexes. In the case of peptides, the number of studied ligands containing histidine or cysteine predominate over the common amino acids and it justifies the separate coverage of the complexes of these residues. The general features of the complex formation reactions between amino acids, peptides and the most common transition elements have already been well clarified and many reviews were cited in the previous volumes of this series. The most recent reviews published in 2003 and 2004 cover the results of some specific types of ligands and also some specific applications of the metal complexes of amino acids and peptides.

Metabolism of copper and its interaction with histidyl imidazole side chains of proteins are believed to be involved in the pathogenesis of neurodegenerative disorders. As a consequence, the interaction of copper(II) with histidine-containing peptides received increasing attention and the results have been reviewed by several authors. One of these works gives an overview on the application of ^{1}H NMR spectroscopy for the investigation of the complexation reactions of copper(II) with peptides of histidine.[3] The other reviews provide an excellent account of the present stage of the role of metal ions in the development of prion and Alzheimer diseases.[4–6] The amino acids and peptides containing the chelating bis(imidazolyl) residues are very efficient and versatile ligands towards transition elements and their complexation reactions were also reviewed recently.[7] The peptides containing the phosphinic acid moiety comprise another interesting group of bioligands and their complexation was reviewed by a special emphasis on their role in the inhibition of zinc containing proteases.[8] The role of aromatic side chains as neutral donor groups for alkali metal cations was also assessed on the basis of theoretical and experimental studies.[9]

Vanadium complexes of different oxidations states are promising candidates for the oral treatment of diabetes and the metal ion is an important constituent of

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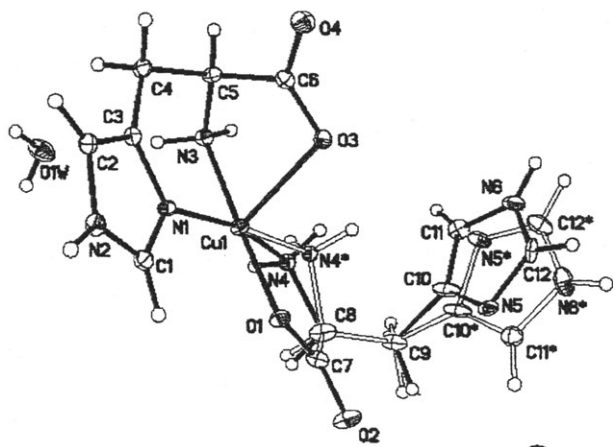
several enzymes. Two reviews have been published in the covered period, including an overview on the complexation reactions between vanadium(IV) and oligopeptides discussing the possible anchoring donor sites,[10] and the speciation in the peroxovanadate-dipeptide systems.[11] The adsorption of amino acids and peptides on minerals[12] and metal surfaces[13] has also been reviewed and these studies are the subject of increasing interest because of possible biochemical and industrial applications.

The various fields for the applications of peptide complexes were enormously increased in the past few years and this subject was nicely highlighted recently.[14] The incorporation of metal binding sites into peptides is an elegant method for the stabilization of peptide microstructures. The most recent review in this field provides examples on the conformationally fixed α-helix-, β-sheet- or various turn/loop-motifs.[15] The use of dinuclear transition metal complexes as auxiliary chromophores was suggested in chiroptical studies. The examination of the CD spectra of the *in situ* formed chiral complexes allows assignment of the absolute configuration and conformational features of the ligands as well.[16]

2. Amino acid complexes

2.1 Synthesis and structural studies

Copper is an essential trace element required by all living organisms. This fact is one of the reasons why interactions between copper(II) and amino acids or derivatives are a continuous focus of interest. Several papers discussing results for complexes of amino acids with copper (II) or with other transition metals, have been published during the past two years.[17–36] New results for a copper(II)-bis(L-histidinato) complex were reported in two papers.[17,18] A single crystal of the neutral five-coordinated complex was analysed by X-ray method. The results show distorted square pyramidal geometry of the complex in which one of the ligands coordinates in a bidentate way, while the other is tridentate. The basic character of the pendant imidazole group and H-bonding interactions of bidentate histidine are suggested as important factors for copper transport in human blood (**1**).[17]



(1)

In solution, EXAFS was used to study the bonding modes in the $[Cu(His)_2]$ complex at pH = 7.3. The results supported symmetric *histamine–histamine* coordination mode of the two ligands in square-planar arrangement. This means that the binding isomer, in which both histidine molecules coordinate to copper(II) ion through their amino-N and imidazole-N atoms, is predominant.[18] In another work, Cu(II) complexes with amino acids Lys and Gly were prepared and

characterized by elemental analysis, infrared spectroscopy and thermogravimetry.[19] A homochiral, two-dimensional neutral coordination polymer was obtained when the complex from the cobalt(II)-3-amino-(*S*)-tyrosine system was isolated.[20] Several papers have presented results for preparation and characterization of zinc(II) complexes with different amino acids including Leu, Asp,[21–23] or bis(glycinato)ferrous hydrochloride.[24] The coordination sphere of the inert chromium(III) was partly[25–27] or completely[27] saturated by coordinating amino acids when Cr(Met)-$(NO_3)_3 \cdot 2H_2O$, $Cr(Met)_2(NO_3)_3 \cdot 2H_2O$, $Cr(AA)_2(NO_3)_3 \cdot 2H_2O$ (AA = Val, Leu, Thr, Phe and Tyr), or $Cr(AA)_3$ complexes were synthesized. The usual bidentate coordination of the amino acids *via* their amino-N and carboxylate-O atoms was found in these complexes by the results of elemental, IR and TG-DTG analysis.[25–27] Solid state complex, $[V(HCys)_3] \cdot 2HCl \cdot 2.5H_2O$, of vanadium(III) with L-Cys was prepared. The characterization of this complex was performed by microanalysis, circular dichroism and IR spectroscopy, as well as room temperature magnetic susceptibility measurements. The results obtained show coordination of each $HCys^-$ ligand through amino-N and carboxylate-O atoms. In solution, progressive oxidation of the complex to oxovanadium(IV) species was found, but some V(III) species were also present after several hours. Antimetastatic, antioxidant and enzyme inhibitory effects (inhibition of neutral endopeptidase) of the V(III) species were demonstrated.[28] The first vanadocene(IV) complexes of α-amino acids, Gly, L-Ala, L-Val, were prepared. All complexes were characterized by elemental analysis, IR, Raman and EPR spectroscopies and coordination of the amino acids *via* their amino-N and carboxylate-O was proposed.[29] IR spectroscopy, thermogravimetry and differential scanning calorimetry were used to characterize and investigate the thermal degradation of the Cd(II) complexes with His or Cys.[30] Results for complexes of amino acids with Pt(II) and Pd(II) can be found in several papers.[31–36] The stereochemistry—both configuration and conformation—of platinum complexes with allylglycine, methionine, S-methylcysteine and their sulfoxides have been investigated by 1-D and 2-D H NMR spectroscopy, molecular mechanics calculations and X-ray crystallography. As representative examples, proton NMR spectra of the equilibrium mixture of isomers of $Pt(MeCys)Cl_2$ (lower trace) and $Pt(MeCysSOH)Cl_2$ (upper trace) are shown in Fig. 1.[31]

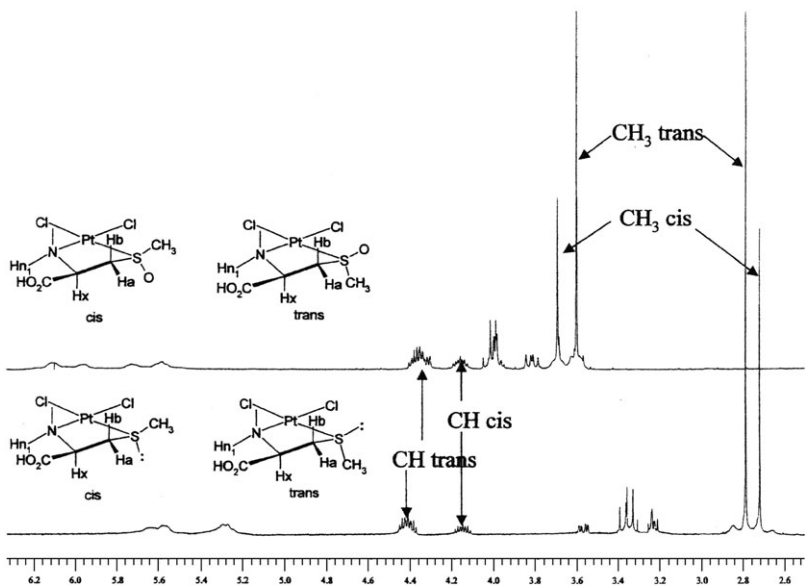
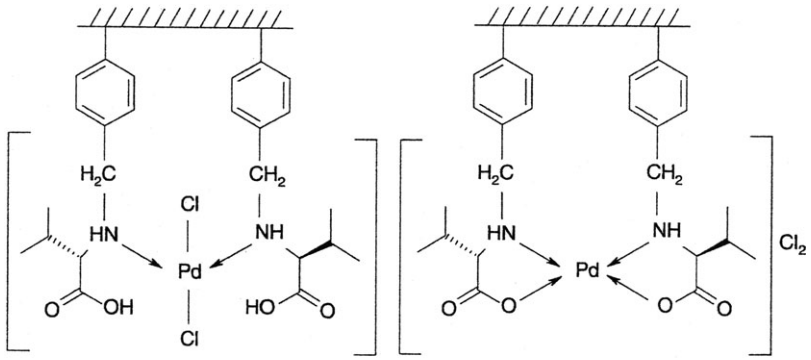


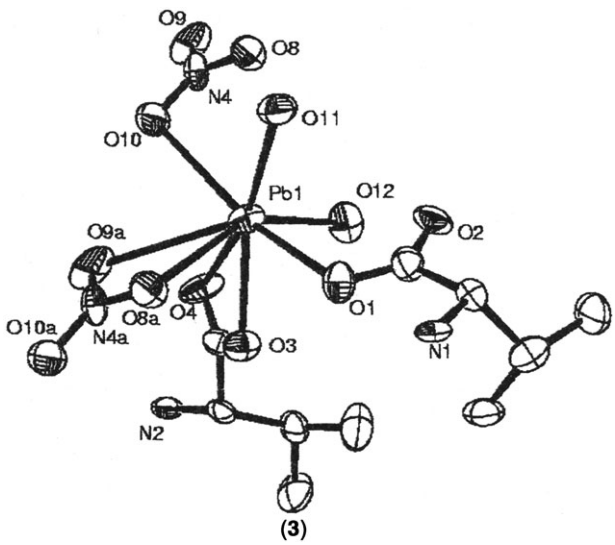
Fig. 1

In addition to the bis-chelated, N,O-coordinated complexes of Ala and Phe with Pt(II), the N,C-coordinated bis-chelated Pt(II) complex of Phe was also synthesized. In the latter complex each Phe ligand coordinates to Pt(II) *via* its amino-N and ortho-C of the phenyl ring.[32] Not only binary, but also ternary complexes with Ala and Phe were prepared and characterized.[33] Polymer-anchored L-Val was used to synthesize polymer-supported Pd(II) complex catalysts for hydrogenation of organic substrates. In the complex formed, L-Val was suggested to coordinate to Pd(II) through either amino-N or both of amino-N and carboxylate-O atoms (**2a**,**2b**).[35]



(2)

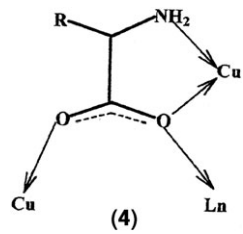
Mono- and bis-complexes of Glu with Pd(II) were prepared and characterized and the former species was found to exhibit antimetastatic properties.[36] A few results for complexes formed between amino acids and ions of p-elements have also been published.[37–40] The first crystallographically characterized Pb(II)-amino acid complex, $Pb(Val)_2(H_2O)_2(NO_3)_2$, was reported in a paper. An eight-coordinated, holo-directed environment was determined for lead(II) in a polymeric solid state structure. The environment of the metal ion is shown in (**3**).[37]



(3)

Several new diorganotin(IV) complexes of L-Cys[38,39] and captopril (N-[(*S*)-3-mercapto-2-methylpropionyl]-L-proline[40] were prepared and characterized. According to the X-ray results obtained for a single-crystal of the complex Me_2Sn(captopril), the Sn atom is five-coordinated, each captopril coordinates to two different Sn

atoms and infinite zig-zag chains are formed.[40] Investigations for amino acid complexes of 4f elements have also been performed during the period reviewed.[41–45] Since lanthanide(III) ions have comparable ionic radii with the calcium(II) ion, and the coordination properties of the former ions are similar to those characteristic for divalent alkaline earth metal ions (especially Ca^{2+}), investigations of lanthanide(III)-amino acid systems can provide useful information about the role of calcium ion in biological systems. By employing absorption spectrophotometry, the interaction of Pr(III) with DL-Ala, L(+)-Arg and β-Ala has been studied in aqueous solution and also in mixed-solvents.[41] A bis complex of Eu(III) with L-Tyr was synthesized and characterized by several analytical techniques,[42] and the bis-glycinato complex of Nd(III) was investigated by X-ray, IR and Raman methods.[43] Tris-complexes of Nd(III) with different amino acids, L-Ala, L-Val, L-Phe, L-Trp, were synthesized and investigated by photoacoustic spectroscopy.[44] A series of new "Ln_6Cu_{24} type" heterometallic clusters of amino acids were synthesized and some of the obtained compounds were characterized by X-ray analysis. Gly and Ala as amino acids and Tb(III), Sm(III), Gd(III) and La(III) as lanthanide ions were involved in this study. The metal skeleton of the clusters can be described as a huge octahedron, which is constructed from 6 Ln(III) ions located at the vertices, plus 12 inner Cu(II) ions located at the midpoints of the edges and 12 additional Cu(II) ions (every 2 of them are connected to a La(III) vertex). Each cluster involves 12 glycinate or alaninate ligands and the coordination mode of the amino acids is demonstrated in (**4**).



(**4**)

Two of the obtained clusters, $[Sm_6Cu_{24}(\mu_3\text{-}OH)_{30}(Gly)_{12}(Ac)_{12}(ClO_4)(H_2O)_{16}]\cdot(ClO_4)_9\cdot(OH)_2\cdot(H_2O)_{31}$ and $[Tb_6Cu_{24}(\mu_3\text{-}OH)_{30}(Ala)_{12}(Ac)_6(ClO_4)(H_2O)_{12}]\cdot(ClO_4)_{10}\cdot(OH)_7\cdot(H_2O)_{34}$, were characterized by a X-ray method.[45] During the past two years numerous papers have presented results for interactions (salt effects) between amino acids and alkali metal ions.[46–50] The adsorption of biomolecules on metal surface can lead to important applications *e.g.* in biomaterials and biosensors. The adsorption of amino acids on metal surfaces,[51–58] namely adsorption of Gly, Val and Lys on silver colloidal particles,[51] D-penicillamine also on silver colloid,[52] α-phenylglycine on silver sols,[53] Lys on gold colloid,[54] Gly on a NiAl (110) surface,[55] Ala on Cu (001) surface[56] and Cys on partically hydroxylated rutile (100) and (110) surfaces,[57] as well as on Cu (110)[58] was investigated during the period reviewed (Fig. 2). In the latter work chemisorption of (*S*)-Cys on Cu (110) was characterized by Fourier-transform reflection absorption infrared spectroscopy (FT-RAIRS) and X-ray photoelectron spectroscopy (XPS). Based on the results, it was concluded that under vacuum, the Cys molecule interacts with the copper surface through the sulfur atom, and the two oxygens of the carboxylate group are equidistant to the surface, while the ammonium group adopts a normal position on the surface. On the contrary, after adsorption in solution, the interaction proceeds *via* the sulfur atom, but the two carboxylate oxygens are not equidistant and the orientation of the ammonium group is not normal to the surface.[58]

Investigations on ternary complexes of amino acids have been in the focus of interest since several decades. As a consequence, numerous papers on metal ion-amino acid/derivatives-"second ligand" complexes have come out during the past two years. Previously, some aromatic diamines, especially 1,10-phenanthroline, 2,2′-bipyridine as "second ligand", were much favoured and during the period reviewed,

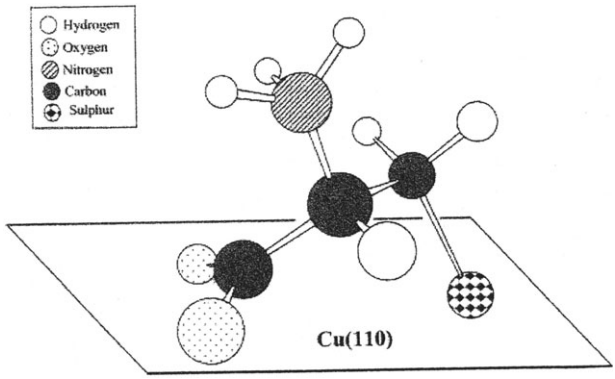
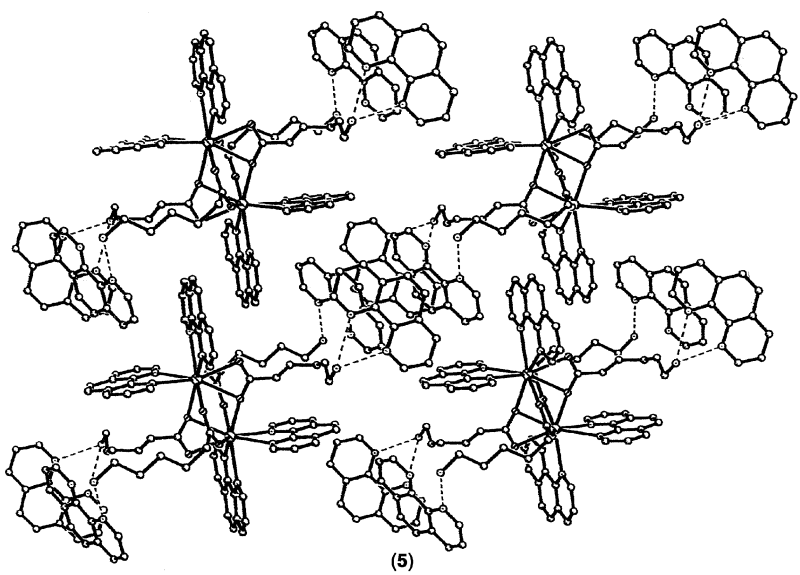


Fig. 2

ternary complexes involving the mentioned diamines, have been also investigated.[59–62] The main goal was the construction of molecular solids with specific supramolecular structures and functions, when ternary complexes of different amino acids with 1,10-phenanthroline and La(III), Eu(III) or Nd(III) were synthesized.[59,60] The effect of the chain-length on the architecture have been studied by three new compounds involving lanthanum(III), 1,10-phenanthroline and 3-aminopropionic acid, 4-aminobutanoic acid or 6-aminohexanoic acid. X-ray results show that, in these complexes, the amino acids exist in zwitterionic form and coordinate to La(III) ions *via* their carboxylate groups. The three compounds are all composed of binuclear units, in which the central La(III) ions are nine-coordinated. However, both the inner coordination sphere and supramolecular structure are modified by the chain length of the amino acid. As a representative example, the two-dimensional supramolecular lamellar architecture of the complex formed with the longest chain 6-aminohexanoic acid is presented in (**5**). The importance of hydrogen bonding and π–π stacking interactions is clearly demonstrated in this structure.[59]

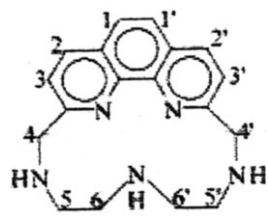


(**5**)

By keeping the former ligands, but using Eu(III) or Tb(III) instead of La(III), new complexes showing photochromic behaviour were synthesized.[60] New chiral ternary complexes of Sn(IV) have been prepared by using Tyr or Phe, plus 1,10-, or 4,7-, or

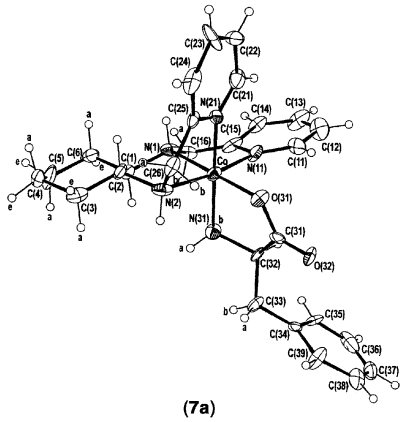
1,7-phenanthroline ligands. The complexes were characterized by IR, UV, ^{1}H, ^{13}C and ^{119}Sn NMR tecniques.[61] The aromatic 8-hydroxyquinoline was used as a second ligand in the prepared and characterized ternary complexes of Co(II), Ni(II) and Cu(II) with Asp and Glu,[63] and in another work with Co(II) with Ala, Val, Leu, Phe, Cys or Glu.[64] Mixed ligand complexes of various metal ions, Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), La(III) and UO_2(VI), with amino acids, Phe, Tyr or Met, as primary ligand and anti-5-nitro-2-furfuraldoxine as secondary one, have been synthesized and investigated by different techniques.[65] Interestingly, evidence was obtained by tandem mass spectrometry for the coordination of the basic side chain of Lys to Zn(II) in some ternary complexes.[66]

Over the years, great interest has focused on the role of metal ions in the active centres of metalloenzymes. Ternary complexes of amino acids are often constructed as structural or functional models of enzymes. With the discovery that manganese is an essential component in active centres of certain enzymes, this element has become the focus of interest. A series of imidazole-containing ternary complexes of Mn(III) have been prepared as models of enzymes containing Mn(III)–His complexation and the importance of the hydrogen bond interactions in the prepared complexes was evaluated.[67] Ternary complexes are used also as models of selective recognition of substrate molecules by metalloenzymes. In a Zn(II) complex of a phenanthroline-containing macrocycle (**6**), the metal ion displays an unsaturated coordination sphere, and as a consequence, can act as a binding site for additional ligand (substrate). Amino acids in their zwitterionic form were found to bind to Zn(II) through their carboxylate functions, but in their anionic form *via* their amino groups. All the results (X-ray and solution equilibrium) confirmed that amino acids containing aromatic pendants were able to form the most stable complexes due to the hydrophobic and/or π-stacking interactions between the aromatic subunits of the substrates and the phenanthroline moiety of the metal receptor.[68]

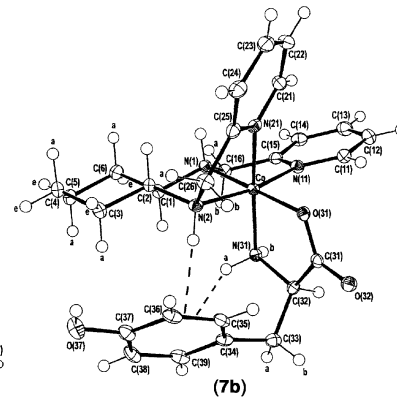


(6)

Redox active molecular receptors were designed to complex and recognize guest amino acids, Lys and Leu. The receptor complexes were Cu(II) and Ni(II) cyclidenes linked by one or two 15-azacrown-5-ethers. With Cu(II) ion, cyclic voltammetry supported the formation of two reversible redox complexes, $Cu^{II/III}$ and $Cu^{II/I}$. Cyclic and differential pulse voltammetric techniques suggested an interaction between the NH_3^+ group of Lys and the crown ether moiety, and also between the carboxylic group and the Cu(II) center,[69] *via* the formation of ternary complexes. Moderate enantioselective recognition of amino acids was achieved also by bis-complex of oxazoline with Cu(II).[70] Again, the formation of ternary complex is the molecular background of the recognition of histamine by Zn(II)-protoporphyrin.[71] Ternary complexes of Co(II) with *N*,*N*′-di(2-picolyl)-1,2-diaminocyclohexane and Phe or Tyr were prepared as simple models for investigation of non-covalent weak interactions that occur and provide the basis of molecular recognition in a complex biological system.[72,73] Interestingly, the aromatic side group of Phe is extended (**7a**), but weak intramolecular NH–π interaction is shown unambiquosly in the tyrosine-containing complex (**7b**).[73]

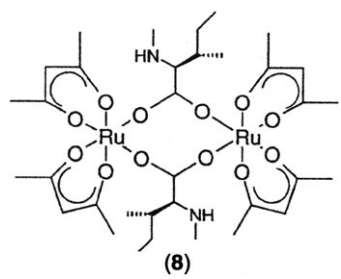


(7a)



(7b)

Ion-pair formation was found as the molecular background of recognition between coordinatively saturated Co(III) complexes, *e.g.* bis-histidinato complex of Co(III), and calixarenes.[74] In another work, recognition of amino acids by water-soluble chiral Mn(III)-salen complex in host-guest interaction was evaluated with the help of ternary complexes prepared.[75] Chiral recognition was studied by using Cu(II) complex of chiral derivatives of bipyridine. This complex was found to form more stable ternary complex with D-Phe compared to L, while the Trp enantiomers were practically indistinguishable.[76] Intramolecular ring stacking between *o*-iodo-hippurate and acyclovir (nucleobase derivative) was found as an important recognition pattern in their ternary complexes prepared with Zn(II), Co(II) and Ni (II).[77] Ru(III)-containing ternary complexes as potential therapeutic nitric oxide scavengers have been synthesized. By reacting the amino acids, Pro and N-methyl Ile, with [Ru(β-diketonato)$_2$(MeCN)$_2$ (CF_3SO_3)], the first examples of paramagnetic μ-amino acidato-bridged Ru(III) dimers were obtained (**8**). Upon treatment of these dimers with base, the corresponding monomers, in which the amino acids form the ''usual chelate'', were formed. [78]

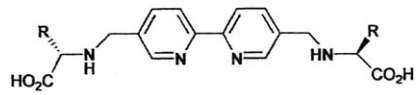


(8)

To obtain additional information about the coordination behaviour of piroxicam (4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzo-thiazine-3-carboxamide 1,1-dioxide), which is an anti-inflammatory drug, its ternary complexes with various metal ions, Fe(II), Fe(III), Co(II), Ni(II), Cu(II) or Zn(II), and Gly or DL-Phe were prepared and characterized.[79]

Investigation of metal complexes of various amino acid derivatives is permanently the focus of interest, because 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 also studied.[80–115] Investigations in this subject have been initiated by various aims, but in many cases, by the biochemical and pharmacological properties of the complexes. They are often prepared as structural or functional models for biomolecules.

Numerous previous results confirm that a substituent at the amino group reduces the affinity of the amino-N atom for metal ions, but it is also true that—depending on the nature of the N-substituent—new types of coordination modes might be achieved with amino acid derivatives. Fe(II), Co(II) and Co(III) complexes of new N-substituted ligands, 5,5′-di(methylene-N-amino acidyl)-2,2′-bipyridyl, where the amino acid is Val or α-Ala (**9**), have been investigated. A clear increase in diastereoselectivity was observed by both upon protonation of the amine groups and upon increasing the steric bulk of the amino acid side chain. The structure of a Co(III)-containing complex was determined by X-ray crystallography.[80]

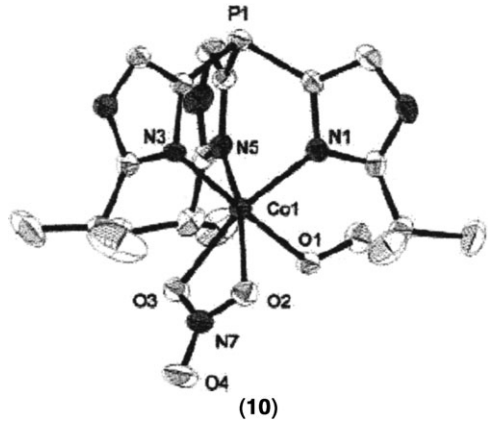


R = i-Pr **1**
R = CH_3 **2**
(9)

N-Substituted derivatives of amino acids Gly, Thr, Ser, Val , Met and also Gly-Gly, were reacted with alkaline earth metal ions and nine hydrated complexes were prepared. The substituent was 6-amino-3,4-dihydro-3-methyl-5-nitroso-4-oxopyrimidin-2-yl group. The results obtained during the structural characterization of the prepared complexes have shown extensive series of hydrogen bonds.[81] Both solution equilibrium and solid state characterization of the complex formed between N-pyrimidine derivative of Met and Mn(II) have been performed. Two different Mn(II) ions having six- and sevenfold coordination environments were found to alternate along an infinite chain in the complex, which can be formulated as $[Mn(H_2O)_4(\mu\text{-}L)_2Mn(L)_2(H_2O)] \cdot 8H_2O$, where L = N-2-[4-amino-1,6-dihydro-1-methyl-5-nitroso-6-oxopyrimidinyl)methionine.[82] Pt(II) complexes of N-substituted amino dicarboxylates were prepared and tested against the leukemia L1210 cell line.[83] The copper(II) complex of an N-substituted unnatural amino acid, (2*R*,1′*S*)-2-(1′-benzyl-2′-hydroxyethylamino)-4-phenylbutanoic acid is the first crystallographically described mononuclear five-coordinated transition metal complex of the type [M(ONO)LL′], where (ONO) is the tridentate amino acid derivative with O-carboxyl, N-amino and O-hydroxo donors, L and L′ are monodentate ligands.[84] The highly stereoselective synthesis of (1*S*,2*S*)-2-amino-1-hydroxyalkylphosphonic acids was achieved by addition of dimethyl phosphite to N-protected aminoaldehydes. Due to the hydroxyl moiety, these new ligands are more potent chelators of Cu(II) and Zn(II) than the structurally related simple aminophosphonic acids.[85] Different spectroscopic techniques were used to characterize Fe(II) and Cu(II) complexes of methionine sulfoxide. The coordination of the carboxylate and sulfoxide groups was suggested by IR results.[86]

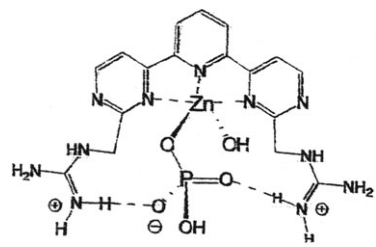
Higher Cu(II)-binding capability was achieved when the amino group of α-Ala was replaced by an oxime moiety. According to the X-ray, IR, Raman and EPR results, a strong intramolecular H-bond stabilizes the two oxime derivatives in *cis*-planar coordination in the bis-complex.[87] Not too many results for Cd(II) complexes of amino acids/derivatives were published, but an interesting carboxylate-bridged dinuclear complex with bis(2-pyridyl-methyl)amino-3-propionic acid was successfully prepared and characterized by X-ray and cyclic voltammetry. The metal ion in the complex is capable of reversible one-electron reduction to Cd(I), but the oxidation to Cd(III) is irreversible.[88]

Since histidine imidazole rings serve as metal-binding sites in many metalloenzymes, various imidazole-containing ligands are often synthesized to mimic multihistidine coordination in biological systems. During the period reviewed, a new water soluble tripodal imidazolyl ligand was prepared and its complexes with Ni(II), Zn(II) and Co(II) ions were synthesized and characterized. Interestingly, the Co(II) complex (**10**) shows structural similarities to the hydrogencarbonate complex of cobalt-substituted human carbonic anhydrase II.[89]



(10)

In another work, Mn(II), Cu(II) and Zn(II) complexes of various bis-imidazolyl ligands were synthesized and five of them were characterized by X-ray crystallography.[90] Additional new multidentate amino acid derivatives and their complexes have been constructed during the past two years.[91–95] Gly or Phe was used as a building block in new tetradentate tripodal ligands and structural mobility of their Zn(II) complexes was investigated by temperature-dependent ^{1}H NMR measurements.[91] Among others, guanidinium and 2-amino-4,5-dihydro-imidazolium auxiliaries as pre-organized recognition sites were attached to the 2,6-di(pyrimidin-4-yl)pyridine framework and their effects in Zn(II) and Cu(II) complexes were investigated both in solution and in solid state. As a result of hydrogen-bonding and charge-pairing, the auxiliary guanidinium groups were found to increase the stability of the Zn(II) complex in the presence of phosphate anion (**11**).[92]

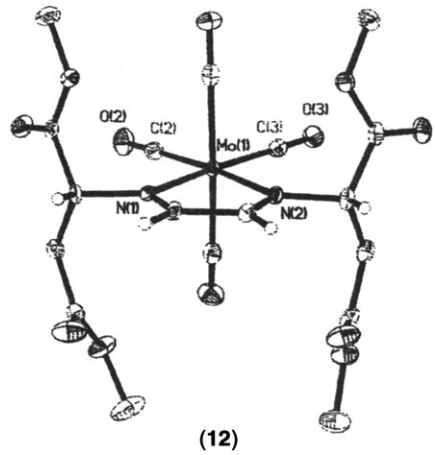


(11)

Phenyltrisalanine and phenylbisalanine as molecular receptors were constructed and their complexation with Eu(III) was investigated by photoluminescence and UV spectroscopy.[94] By heating solutions of ester-protected amino acids (H-L-Ala-OEt, H-β-Ala-OEt, H-L-Val-OMe, GABA-OMe, H-L-Asp(OMe)OMe) and glyoxal in the presence of $M(CO)_4(pip)_2$, where M = Mo, W, new compounds of molybdenum and tungsten were constructed. Crystal structures of molybdenum compounds formed with the derivative of Asp were determined. Among them, the molecular structure of the (*R, S*) isomer is presented in (**12**).[95]

Numerous binary and also ternary complexes of amino acid-based Schiff bases with various metal ions such as Cu(II),[96–102] Ni(II),[101,103] Zn(II),[100,102] Fe(III),[100,104] Fe(II),[105] Mn(II),[102] Mn(III),[106] Co(II),[101,102] V(IV),[107] La(III) and Ce(III),[108] Sn(IV),[109,110] K(I) and also some other monovalent cations,[111] have been prepared during the past two years. Almost all kinds of natural amino acids have been used to synthesize Schiff bases.

The new compounds obtained show different biological effects and are all very effective metal ion complexing agents. Their complexes are considered as good models for a number of important biological systems. As mentioned already in this

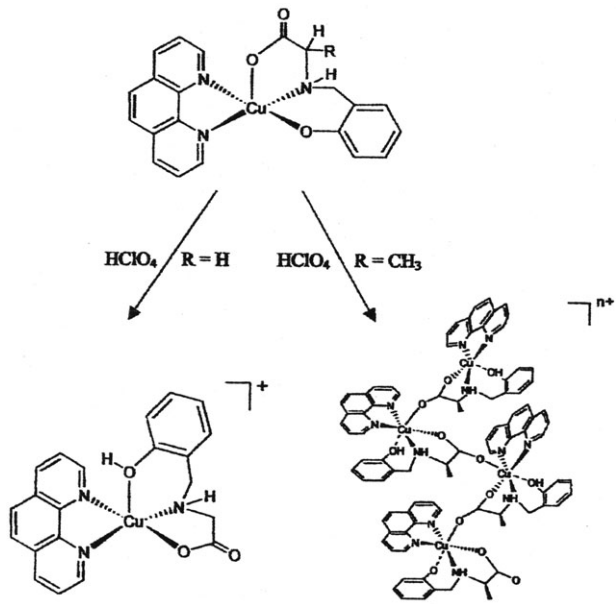


(12)

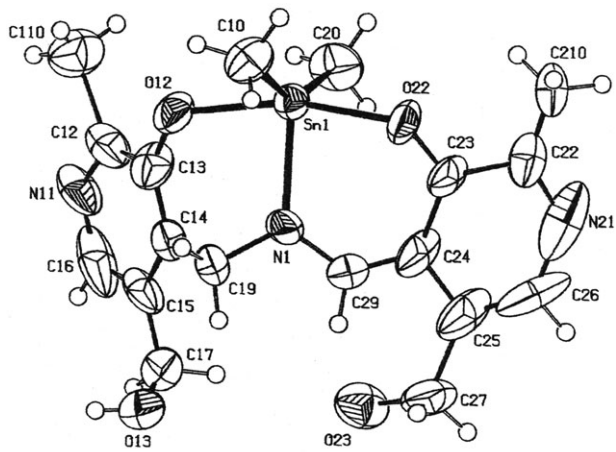
section, His has outstanding importance in metal-binding of biological systems. The Cu(II) complex of a new Schiff base derived from His, was synthesized and characterized by X-ray crystallography.[96] A vitamin B6 model was prepared by synthesizing Cu(II) ternary complex, in which one of the ligands is a Ser derivative Schiff base.[97] Interesting new Cu(II) ternary compounds of reduced Schiff base ligands and 1,10-phenanthroline were prepared and some of them were characterized by X-ray crystallography. On the other hand, spectroscopic results suggested that protonation of the neutral mononuclear complexes caused structural changes at the Cu(II) centers and, depending on the amino acid side-chain (–H, or $–CH_3$), different types of solid state products were formed (Scheme 1).[99]

Carboxylate-bridged Fe(III) dinuclear complexes, as models for enzymes that activate molecular oxygen in biological systems, were synthesized and characterized.[104] A new complex involving amino acid-based Schiff base and iron in 2+ oxidation state was also prepared.[105] Another synthetic work resulted in a new series of Mn(III) complexes of L-Ser-, L-Met- and L-Cys-based Schiff bases.[106] Extensive work has been performed on V(V)-N-salicylideneamino acidato systems, where sulfur-containing amino acids, L-Cys, D- and D,L-penicillamine have been used to construct the new ligands.[107] Diorganotin(IV)-promoted deamination of Val or Gly by pyridoxal (biologically active form of vitamin B_6) resulted in the formation Schiff base complexes with SnR_2, where R = Me, Et. Crystals of two complexes, in which the ligand is coordinated to the metal ion through the O atoms of the two deprotonated phenolic hydroxyl groups and the iminic N atom, and the metal exhibits distorted square pyramidal coordination, were determined by X-ray analysis. Both complexes show intense antibacterial activity. As an example, the structure of the complex formed with $Sn(Me_2)^{2+}$ is shown in (**13**).[110] The catalytic potential of ruthenium complexes in organic synthesis is well known. Frequently, half-sandwich (η^6-arene)ruthenium complexes are used as pre-catalysts. The crystal structures and catalytic properties of newly synthesized diastereomeric half-sandwich complexes of Ru(II) and Os(II) differing only in the metal configuration, have been studied. In this work, different α-amino acid esters, such as methyl phenylalaninate, methyl phenylglycinate, and optically active amines, were reacted with salicylaldehyde to obtain the chiral ligands.[112]

Sarcosine, N-methylalaninate and ethylenediamine-*N*,*N*′-diacetic acid have been used to create half-sandwich complexes with iridium, ruthenium, rhodium, and phosphine-containing complexes with palladium and platinum.[113] In addition to several monometallic half-sandwich complexes, a heterometallic lysinate-bridged complex of iridium and rhodium (**14**) was also prepared.[114]

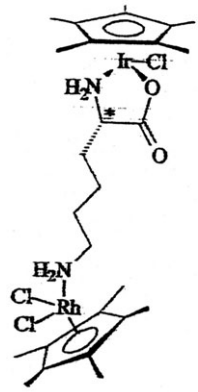


Scheme 1



(13)

Various types of half-sandwich complexes (including monomeric, dimeric, trimeric ones) of *R*-3-(3-pyridyl)alanine were synthesized and characterized in another work (one example of the prepared complexes is shown in (**15**)).[115]



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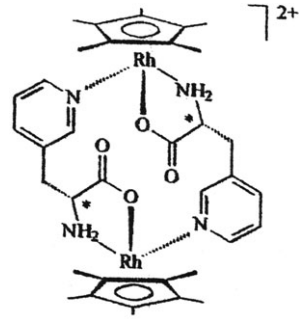
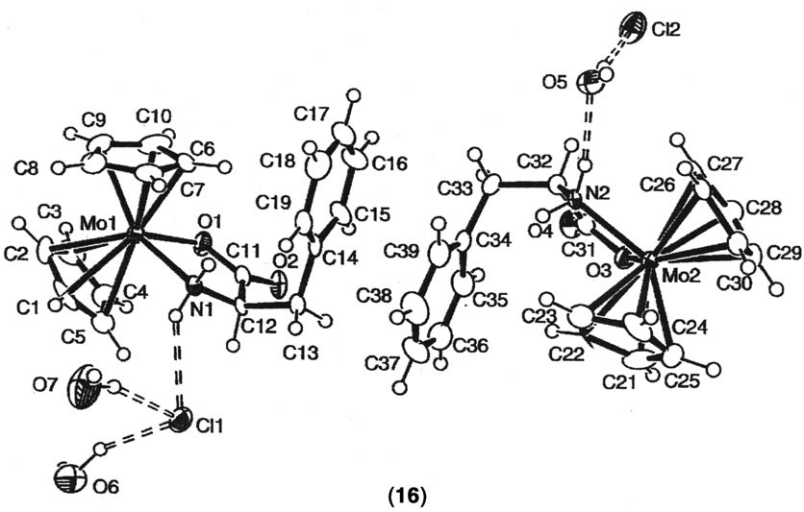


(15)

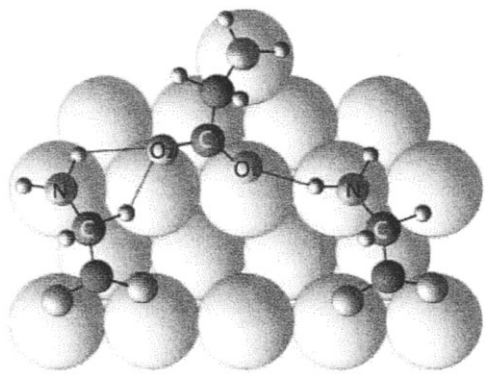
Due to their antiproliferative properties against diverse animal and human tumors, metallocene compounds have special biological importance. Molybdocene-amino acid compounds of D-Phe, DL-Leu and DL-Val were synthesized and characterized by X-ray method. Significant H-bonding, plus in the phenylalaninato complex π-stacking between the phenyl rings was found (**16**).[116]



(16)

Investigations of metal ion-biomolecule complexes in the gas-phase provide valuable information because the complexes are free molecules and therefore are not affected by local environment (solvent) that can stabilize or destabilize them. Thus such gas phase results are very useful for many reasons, for example, for understanding the role of metal ions in initiating biochemical processes. As a consequence, during the past two years, numerous papers have discussed experimental results for different metal ion-amino acid complexes in the gas phase and also for theoretical calculations.[117–141] The various techniques of mass-spectrometry are useful in this field and have also been used in various combinations. Complexes of Cu(I) and Ag(I) with Gly,[117] and the reactivity of the complexes $CuGly^+$ and $AgGly^+$ towards the neutral small molecules, CO, D_2O and NH_3,[118] have been investigated. A high degree of chiral recognition of di-*o*-benzoyl-tartaric acid dibutyl ester, *via* the formation of its ternary complexes with Cu(II) or Zn(II) and L-Trp, was achieved in the gas-phase by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry.[119] A quadrupole ion trap mass spectrometer was used to study the dissociation patterns of Cu(II) complexes with various ligands and the preference of Cu(II) to remain coordinated to a particular functional group was found to follow the trend: thioether > amine > imidazole > pyridine > ether.[120] Electrotopological state results for the second stepwise formation constants of binary and ternary Cu(II) complexes with α-amino acidate ligands suggested that the stability of the "second" chelates depends mainly upon variations in the coordination tendency of the carboxylate groups.[121]

Formation of intermolecular hydrogen bonds between glycinate ions on a Cu (110) surface (**17**) was supported by theoretical results.[122]

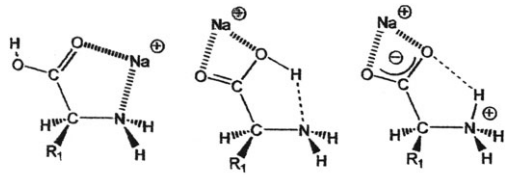


(17)

Quantum chemical calculations were used to explain the observed enantiodifferentiation in the complexation of α-amino acids to chiral Cu(II) complexes.[123] Results of calculations for the interaction of Gly with Zn(I) and Zn(II) ions, and further with one or even two H_2O molecules in the gas phase have been published in a recent paper.[124] Theoretical calculations also for cobalt(III)-alaninate,[125] palladium(II) and platinum(II)-methioninate and histidinate[126] have been performed and published.

Interactions between alkali metal cations and amino acids have received much interest, and many experimental and theoretical investigations have been devoted to such systems during the past two years. Gly has been chosen several times as the prototype of amino acids,[127–129] but almost all of the essential amino acids have been involved in the investigations cited.[130–138] As it is already known, Gly in solution exists in zwitterionic form, while in the gas phase its neutral form dominates. On the other hand, interaction with a metal cation can stabilize the zwitterionic form. The vibrational spectra of the Na(I) complexes of Gly and Pro in the gas phase suggested the preference of different bonding isomers with the two ligands. While the salt-bridge isomer (**18SB**) was found to dominate in the case of Pro-Na^+, the charge

solvation by chelation of Na^+ between nitrogen and the carbonyl oxygen seemed to be the most favourable for Gly-Na^+ (**18CS1,CS2**).[127]



(18)

In numerous papers, results for interactions between alkali metal cations and amino acids containing different side chains have been discussed[130–137] and, in some cases, the effects of side-chains have been evaulated.[130,134,135] By choosing aromatic amino acids, the effect of the non-covalent cation-π interaction on the stability of some alkali metal ion-amino acid complexes has been investigated.[134,135] The distance between the amino and carboxyl functions is different in α-Ala compared to β-Ala, which leads to some differences in their cation-binding capability.[138] A few papers have been published about interactions of alkali earth metal cations with Gly by using quantum chemical methods.[139,140]

Because the imidazole function plays a crucial role in the metal-binding of many biological systems, its interactions with a variety of metal ions have been studied by both experimental (guided ion beam mass spectrometry) and theoretical methods.[141]

2.2 Solution studies

An enormous amount of equilibrium data for metal ion-amino acid systems has been determined during the past several decades. But numerous new data have been again appeared during the period of this coverage. In addition to pH-potentiometry, different spectroscopic methods and calorimetry were used to determine the stoichiometry and stability of complexes formed in the studied systems. In many cases, the studied complexes have been characterized both in solution and in the solid state. Several binary systems like Cu(II)- and Ni(II)-Gly, -L-Ala, -L-Val, -L-Leu, -L-Ile, -L-Ser, -L-Phe and -L-Met,[142] Cu(II)- and Fe(II)-Glu, -Leu, -Trp[143] and Ni(II)-Gly[144] have been investigated in mixed solvents and the results obtained have been compared with those in water. Ionic strength dependence of the formation constants have been determined in the systems Mo(VI)-His,[145] Mo(VI)-Cys,[146] U(VI)-Glu,[147] V(V)-Tyr,[148] V(V)-Gly.[149] By using calorimetry, several new thermodynamic data have been calculated for the complexes formed in the Zn(II)-Gly,[150,151] Zn(II)-Val,[152] Zn(II)-His,[153] Ni(II)-Val[154,155] systems and also for complexes of L-His with first-row transition metal ions.[156] Interaction of Co(II), Ni(II), Cu(II) and Zn(II) with L-Ser have been investigated by performing potentiometric titrations at various temperatures and ionic strengths.[157]

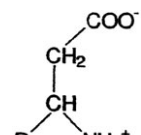
Only a few equilibrium data have been previously published for systems in which the metal ion-amino acid interaction is quite weak and/or there is strong competition between the hydrolytic processes of metal ions and the complex formation with amino acids. Numerous new data have appeared for these types of systems during the past two years. V(III) has a strong affinity both for oxidation and hydrolysis. Complexation of this metal ion with L-Ala and L-Asp in aqueous solution under nitrogen was studied by potentiometric and different spectroscopic (UV-Vis, CD) methods. Only mononuclear species were found with L-Ala, while both mono- and various dinuclear species were suggested to form with L-Asp.[158] Stability constants have been determined for the complexes of V(V) ion with some sulfur-containing amino acids, L-Cys, N-acetyl-L-Cys and DL-penicillamine in a recent work.[159] Some new formation constants and

thermodynamic data have been published for complexes of methylmercury(II) (CH_3Hg^+) with a series of ligands, including Gly and Cys.[160]

Generally, typical hard metal ions like lanthanides, aluminium(III), bismuth(III), lead(II) and especially ions of alkali or alkali earth metals do not form high stability complexes with simple amino acids. In spite of this, various aspects have motivated studies on such systems and several new results have come out during the period reviewed.[161–171] A speciation study for Al(III)-Glu system has suggested that Glu forms more stable complexes than simple "glycine-like" amino acids, which results in the increased capacity of glutamate to maintain Al(III) ions in solution under normal dietary conditions.[161] Both solution and solid state investigations on the Bi(III)-L-Cys system have been performed and stability constant for the species having the stoichiometry $[Bi(H_2L)]^{3+}$ has been determined.[162] Some sulfur-containing amino acids have been involved in the solution equilibrium study, in which their complexation with Tl(I) has been investigated.[163] Stability constants for Pb(II) complexes of Val[164] and Lys[165] have been published. In the latter work, Ag(I) and Ca(II) complexes have also been studied.[165] First of all, carboxylate-coordinated complexes were suggested in the dibutyltin(IV)-amino acid system, but evidence for the coordination of the amino-N atom in the solid state complexes was also obtained.[166] Both the ionic strengths and the temperature dependence of stability constants of the complexes formed between Pr(III), Nd(III), Eu(III), Gd(III), Dy(III), Ho(III),Yb(III) and Gly or Thr, have been investigated. By using the results obtained, thermodynamic parameters have also been calculated.[167] Not only solution equilibrium studies, but also solid state characterization of the complexes formed in the Ce(III)-L-Phe system has been performed.[168] Interestingly, some new equilibrium data have been published for Be(II)-His and Be(II)-Cys,[169] as well as for Mg(II)-L-Arg and Ca(II)-L-Arg[170] complexes, and also for alkali metal complexes of cystine.[171]

Although glycinamide is not naturally occurring, it is often studied as a model ligand. A unified method for treatment of potentiometric and polarographic data has been developed and applied for determination of stability constants for Cd(II)-glycinamide complexes.[172]

A lot of investigations for the metal complexation of different amino acid derivatives in solution have been performed. In many cases both the stoichiometry and stability of the complexes formed with derivatives differ from those formed with the parent molecules. Although the usual "β-amino acid-type" 6-membered (N,O)-chelate was found to form in Cu(II) complexes of the β-substituted-β-amino acids (**19**), size-dependent steric effects of the R substituents (R = hydrogen, methyl, ethyl, isobutyl, isopropyl, cyclohexyl, 1-ethylpropyl, *tert*-butyl) on the stability of complexes have been identified and quantified by using pH-potentiometry and EPR spectroscopic methods.[173]



(19)

Not only do the numerical value and/or ratio of stepwise stability constants change in the case of donor atom-substituted (N-and/or O-substituted) derivatives, but also the equilibrium models change. The situation is even more complicated if an additional donor(s) is(are) involved in the side chains. Only a potentiometric method was used to determine the stability constants of the complexes of N-(carboxymethyl)aspartic acid with Mn(II) and Cu(II),[174] as well as with Co(II) and Ni(II)[175] formed in aqueous solution at different ionic strengths. Potentiometric, EXAFS and IR spectroscopic techniques have been combined to investigate the speciation and equilibria in the Cd(II)-N-(phosphonomethyl)glycine (=glyphosate) system. It was

found that, depending on the pH and on the metal to ligand ratio, mono- and bis-chelated complexes can be formed and all three donor groups of the completely deprotonated ligand (amino, carboxylate, phosphonate) are coordinated to the Cd(II) ion *via* joined 5-membered chelates.[176] The N-atom of the Val derivative cannot coordinate to Zn(II) ion in the studied N-pyrimidine derivatives of this amino acid and only low stability monomeric complexes were found in this system in aqueous solution.[177] Previously, only very few data were published for microspecies formed between metal ions and amino acids/derivatives. Recently, an EPR study have been performed on some systems, containing Cu(II) and N-substituted bis(aminomethyl)phosphinate type ligands, for determination of the formation constants and EPR parameters relating to the various detected microspecies.[178] In another work, N-bromoacetyl-(*S*)-phenylalanine methyl ester or N-bromoacetyl-(*S*)-tryptophan as pendant arms were attached to 1,4,7-triaza-cyclononane or 1,4,7,10-tetraazacyclododecane. The stability constants of the complexes formed between these synthesized new ligands and Zn(II), Cd(II) and Cu(II) ions were determined. Although the chiral centre in each pendant arm makes it possible to form two diastereomers of each metal complex, the NMR data suggested that only one diastereomer exists.[179] Previous studies have been continued when stability constants and thermodynamic parameters for the binary complexes of Co(II), Ni(II), Cu(II) and Zn(II) with tricine (N-[tris(hydroxymethyl)methyl]glycine) at various ionic strengths and in different solvent mixtures have been determined.[180]

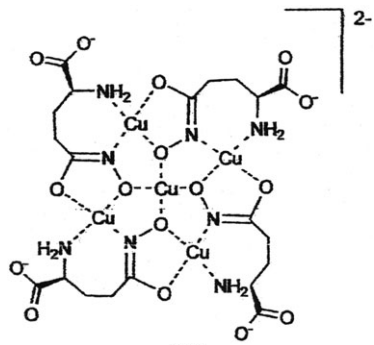
Hydroxamic acids (containing the hydroxamic acid function, [–CON(R)OH] are able to form a five-membered hydroxamate-type (*O*,*O*)-chelate and are known as good chelating agents for hard metal ions. However, in the case of aminohydroxamic acids, when the carboxylic groups of amino acids are modified to hydroxamic ones and the R substituent of the hydroxamic-N- is hydrogen, the coordination *via* amino-N and hydroxamate-N is also possible. As a consequence, these ligands are good chelators also for non-typical hard metals, such as Cu(II), Ni(II), Zn(II). Depending on the conditional stability of different types of chelates, either hydroxamate, (*O*,*O*)-type, (*N*,*N*)-type coordination mode or coordination *via* the amino-N and hydroxamate-N atoms exist. If, however, the stability of these two chelates is comparable, both of them are coordinated and, because of steric reasons, this results in the formation of polynuclear complexes. During the period reviewed, solution equilibrium studies for Cu(II)-α-alaninehydroxamic acid and -aspartic-β-hydroxamic acid,[181] -glutamic-γ-hydroxamic acid[182] systems, as well as for Cu(II), Ni(II) and Zn(II) complexes of succinylhydroxamic derivatives of Pro and Phe,[183] have been performed and interesting new Cu(II)-containing metallocrowns† have been identified by ESI-MS. As a representative example, the structure of 12-metallocrown-4 formed in the Cu(II)-(*S*)-glutamic-γ-hydroxamic acid system is shown in (**20**).[182]

Both solution equilibrium and X-ray results have been published for Zn(II) complexes of two uncommon phosphonic derivatives of Glu. In contrast to the majority of simple aminophosphonic acids, no tendency for cyclization upon the action of Zn(II) has been observed with these two ligands.[184] Some diorganotin(IV) complexes of captopril, {N-[(*S*)-3-mercapto-2-methylpropionyl]-L-proline} have been studied not only in solid state,[40] but also in solution.[185]

To investigate the relationship between antimicrobal activities and the formation constants of Cu(II), Ni(II) and Co(II) complexes with three Schiff bases, which were obtained by the condensation of 2-pyridinecarboxyaldehyde with DL-Ala, DL-Val and DL-Phe, potentiometric measurements were performed. Based on the results, square-planar, tetrahedral and octahedral structures were proposed for the Cu(II), Ni(II) and Zn(II) complexes, respectively, and the correlation between the structure

† Metallocrowns are inorganic analogues of crown ethers and can be obtained by conceptually replacing methylene carbon atoms of the parent ether by a transition metal, and a heteroatom, such as nitrogen, bound in the metal's first coordination sphere.

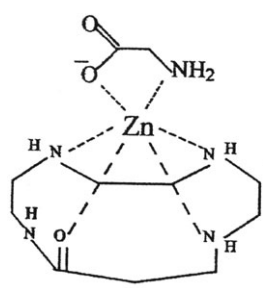
and biological activity was evaluated.[186] A few new equilibrium constants for the species involving one alkaline ion, Li^+, Na^+ or K^+, and penicillamine disulfide have also been published.[187]



(20)

There is no doubt that ternary equilibrium systems are often much more complicated compared to their binary ones, but because, in many cases, the ternary complexes are better models of various natural compounds, for example metalloenzymes, their investigation is of great importance. As a consequence, numerous new data, especially stability constants and a few thermodynamic data have been published during the period covered.[188–212] In a few cases, both ligands are amino acids in the ternary complexes. For example, Met and Cys were the two ligands when the stability constants for their Fe(III) and Cr(III) ternary complexes were determined.[188] In another work, His was used as primary ligand and Ala, Val or Leu,[189] as well as Ala, Trp or Tyr[190] as secondary ligands, and their complexes with Pr(III) and Nd(III) studied. By using the stability constants and also the spectral results obtained, the various ligand-ligand interactions have been evaluated.[189,190] Again, L-His was the primary ligand and D-Orn is the secondary one when their ternary complexes with Cu(II) were studied by using EPR spectroscopy.[191] Stability constants for ternary complexes of V(IV) with L-His or Asp or Glu as primary and some imidazole (2-methylimidazole, 2-ethylimidazole or imidazole) as secondary ligands, have been determined.[192,193] In several additional ternary systems, only one of the ligands was an amino acid, while the second complexing agent was another type of biologically important molecules, such as guanidinoacetic acid,[194] folic acid,[195] 2′-deoxyguanosine-5′-monophosphate,[196] tricine (N-[tris(hydroxymethyl)methyl]-glycine)[197,198] or cephalexine.[199]

Molecular/chiral recognition problems have remained in the focus of interest during the past two years.[200,201] A new artificial receptor, 1,4,8,11-tetraazacyclotridecane-5-one and its complex with Zn(II) was found to be extremely efficient in the recognition of Gly. The recognition is based on the formation of high stability ternary complex (**21**).[200]



(21)

Stability constants for Cu(II), Co(II), Hg(II) and Pd(II) complexes of a macrocyclic ligand incorporating 1,10-phenanthroline unit and for their ternary complexes with different α-amino acids have been determined.[201] Optically active *cis* β-folded organocobalt-salen complexes with enantiomerically pure α-L-amino acids were characterized both in solution and solid phase.[202]

As this is traditional, ternary complexes of amino acids with multidentate amines, polycarboxylates, amino/polyamino polycarboxylates have been studied in numerous laboratories,[203–211] For example, 1,10-phenanthroline,[203] NTA,[204–206] DTPA,[207,208] CPTA (*cis*-1,2,3,4-cyclopentane tetracarboxylic acid),[209] oxalate[210] and also some newly synthesized polyamino-polyamides[211] have been involved in such studies.

Almost all essential amino acids, and also some dipeptides have been studied in work in which stability constants for Pd(II)-containing ternary complexes of 2-picolylamine (primary) and selected bio-relevant ligands (secondary) were determined.[212]

2.3 Kinetic studies

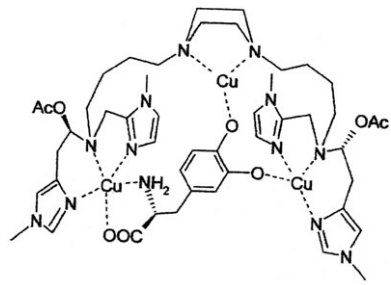
The kinetics of oxidation of different amino acids, involving their interaction with metal ions have been intensively studied during the period covered.[213–243] Namely, oxidation of DL-Met by a Cu(III)-containing compound, ditelluratocuprate(III) in alkaline medium,[213] or L-Pro by diperiodatocuprate(III) also in alkaline medium.[214] MnO_4^- was used as an oxidizing agent in numerous studies.[215–219] L-Val was oxidized by MnO_4^- in a Cr(III)-catalysed reaction in alkaline solution,[215] Gly, Val, Leu, Ile, Phe were oxidized in acidic solution by MnO_4^-,[216] oxidation of Lys by MnO_4^- was performed in the absence and presence of sodium dodecyl sulfate in acidic solution.[217] Oxidative decarboxylation of Lys by MnO_4^- in acidic medium was studied also in another work.[218] Thr was oxidized by alkaline MnO_4^-,[219] and manganese(III) oxidized Cys to cystine under anaerobic condition in aqueous acetic acid.[220] In many cases, V(V) were used as the oxidizing agent.[221–224] Cyclic voltammetry was used to study the V(V)-Cys system. In addition to the characterization of the redox reactions, the stoichiometry and stability constant of V(V)-Cys bis-complex [ML_2] was also determined.[221] Micellar catalysis of a large number of systems, including V(V)-amino acid (Met, Pro, Ala),[222–224] has been the area of several recent investigations because of the similar behaviour of macromolecules and enzymes. In several cases, Cr(VI) was used as the oxidizing agent.[225–227] The kinetics of the oxidation of DL-Met to sulfoxide by Cr(VI) has been studied in aqueous acetic acid in the presence of EDTA, and the Cr(VI)-EDTA complex was suggested as the active electrophile in this reaction.[225] In other cases, diperiodatonickelate(IV) was used as the oxidizing agent in alkaline medium, and L-Leu,[228] L-Pro[229] and L-Glu[230] were oxidized. The kinetics of micellar catalysis of Cys by hexacyanoferrate(III) has been investigated,[231] while in another work alkaline dihydroxydiperiodatoargentate(III) was used to oxidize L-Asp.[232] Oxidative degradation of DL-Met by Ce(IV) in aqueous perchloric acid solution has been studied by spectrophotometry.[233]

In many cases, the metal ion was not the oxidizing agent in the reactions, but formed a reactive transient species with the amino acid.[234–245] Most frequently, Ru(III)-catalyzed oxidation of different amino acids, Gly,[234] Ala,[234] Leu,[235] Phe,[236] Pro,[237,238] Asn[239] has been studied, but Ir(III)-catalyzed oxidation of Asp[240] and oxidation of Cys, catalyzed by macrocyclic cobalt complex,[241] have been also investigated.

Ratio-dependent oxidation of Cys by Cu(II) was studied and evaluated. Up to a ratio 0.45:1 of Cu(II):Cys, the Cu(II) ions are reduced to Cu(I) by the stoichiometric formation of cystine. The Cu(I) produced in this way is complexed by the excess of Cys. Trace amounts of Cu(II), exceeding the ratio 0.45:1, induces fast and complete oxidation of the Cu(I)–Cys complex to cystine with concomitant production of Cu(0).[242] Investigations have been carried out to study the reduction of a Fe(III)-Schiff base complex by L-Cys · HCl, and kinetic data and mechanism of the reaction have been determined.[243] In another work, mechanisms by which Cys can inhibit or

promote the oxidation of a low density lipoprotein by copper(II) have been investigated.[244]

Amino acids such as Gly, Ala, Asp, hydroxyproline, as bidentate ligands, were found to catalyse the oxidation of lactic acid by Cr(VI).[245,246] On the other hand, chromium(II) complexes of natural amino acids have been used succesfully to reduce α-amino acid precursor oximes,[247] or prochiral ketones[248] with high conversion and measurable enantiomeric excess. Remarkable enantio-differentiation in catalytic oxidation of L- and D-Dopa (2,4-dihydroxyphenylalanine) was achieved by a trinuclear Cu(II) complex of a histidine derivative multidentate ligand. The suggested interaction between the substrate and the catalyst complex is demonstrated in (**22**).[249]



(22)

Dinuclear Zn(II) complexes of α-ω-bis(dipicolylamino) acid derivatives of Orn and Lys were found to accelerate phosphodiester bond cleavage of a ribonucleotide model substrate.[250] The acid-assisted dissociation of three ternary complexes of Co(III) formed with ethylenedibiguanide as primary and an amino acid, Gly, α-Ala or Val, as secondary ligands, have been investigated, and the following reactivity order of the complexes was found: Val < α-Ala < Gly.[251] The anation reaction of L-Orn with Cr(III) was found to follow an associative interchange mechanism.[252]

Although platinum complexes with amino acids and their derivatives have attracted the attention of inorganic and coordination chemists in the past, some new results have also appeared during the past two years. *Trans*-[Pt(L-Ser)$_2$] and *cis*-[Pt(L-Ser)$_2$] complexes have been prepared and characterized by X-ray diffraction, and[195] Pt NMR and HPLC techniques used to study the reactivities of the complexes. Their reactivities towards HCl were found to be different, as the coordinated carboxyl oxygen atoms of the *trans* isomer could be detached faster than those of the *cis* isomer.[253] Palladium(II) complexes are often studied as models for the platinum(II) analogues. In a recent publication,[254] thermodynamic, kinetic and structural results for ternary complexes of Pd(II) with bis(pyridin-2-ylmethyl)-amine (bpma) tridentate and several monodentate ligands have been published. Among the monodentate ligands, several N-acetyl amino acid derivatives, AcHis, AcHm, AcLys and AcMet have been involved in the investigations. Although the thermodynamic stability of the ternary complexes formed between $[Pd(dpma)]^{2+}$ and one of the N donor monodentate ligands (*e.g.* cytidine, AcHm, AcHis) is higher compared to the stability of the complex with the thioether ligand, AcMet, the trend of the reaction rate of the substitution reactions is just the opposite. The reaction of AcMet is much faster than that of the N donor cytidine. Ligand substitution reactions of chloromethyl(aquo)cobaloxine with aromatic and aliphatic N donor ligands, Hm, His, Gly and ethylglycine ester have been studied by spectrophotomeric techniques[255] as a function of pH at constant temperature and ionic strength. The kinetics of resolution of *rac*-Phe by stereoselective complexation to a Co(III) complex with a chiral ligand N-carboxylmethyl-N-pyridylethyl-leucine, $[Co(cpel)(CO_3)]^-$, has been investigated. The dynamics for coordination selectivity have been examined by

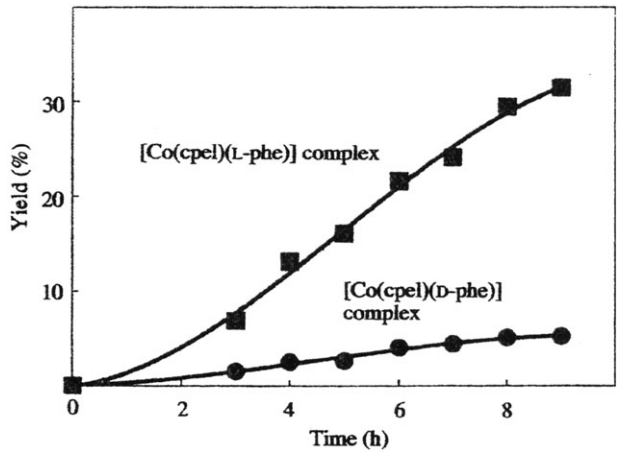
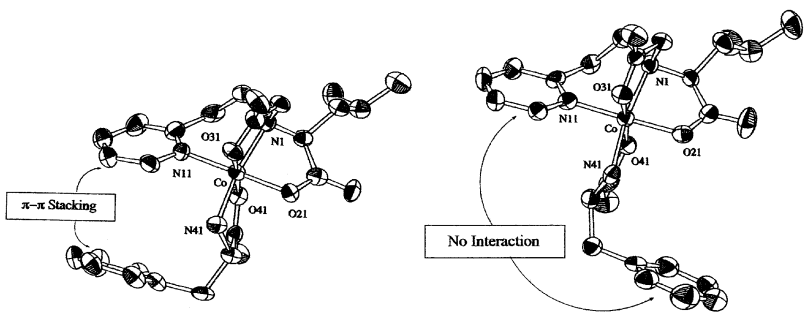


Fig. 3

means of the competitive coordination of racemic Phe to the chiral cobalt complex $[Co(cpel)(CO_3)]^-$ at pH 5 in aqueous solution. A chiral HPLC column was used to measure the formation rates. As shown in Fig. 3, the ratio for the formation rate of the complex with L-Phe to the D-complex is about 6. However, if *rac*-Leu was used instead of *rac*-Phe, the selectivity for [Co(cpel)(L-Leu] to [Co(cpel)(D-Leu)] was only 1.1:1. This result shows that the selectivity for chiral recognition is reduced in a system without possibility of π–π stacking. This conclusion has been supported unambiguously, by X-ray results obtained for the complexes with L-Phe (**23a**) and D-Phe (**23b**).[256]



(23a) **(23b)**

Different reactions were observed when one of the three studied metal alkoxides, $Ti(OEt)_4$, $Al(OBu^s)_3$ and $Zn(OBu)_4$ were reacted with Lys. Reaction of $Ti(OEt)_4$ with Lys resulted in the formation of $Ti(OEt)_3$(lysinate), in which the ligand is coordinated to the metal ion *via* its amino-N and carboxylate-O atoms, by forming the ''usual'' 5-membered chelate-ring. Contrary to that, $Al(OBu^s)_3$ was found only to catalyze the formation of 3-aminocaprolactam and no substitution product was observed. $Zn(OBu)_4$ and Lys produced both reactions.[257]

Mannich reactions of chelated amino acids have also received some attention during the past two years. Reactions of a series of bis(α-amino acidato)copper(II) complexes with formaldehyde and acetamide have been investigated, and the effects of different α-substituents on the Mannich reactions have been evaluated.[258]

The kinetics of thermal decompositions of several ternary complexes formed between Zn(II), Thr and acetate,[259] or between Gd(III), Tyr and Gly[260] have also been

studied in recent works and the mechanisms determined. The fragmentation of metal ion-amino acid complexes in the gas phase may provide valuable information relating to properties of their liquid phase counterparts or properties of various biomolecules, including metalloproteins and metalloenzyme active sites. In this subject, in a recent paper, fragmentation mechanisms of Cu(I) complexes of Gly in the gas phase have been studied.[261]

To understand the concept of how biomolecules interact with the surfaces of biomedical implants (molecular biorecognition), the reaction of DL-Pro on O_2-annealed (stoichiometric) and O_2-defected (sub-stoichiometric) TiO_2 (001) single-crystal surfaces has been investigated. According to the results obtained, Pro does not seem to decompose on a stoichiometric surface, but on the defected surface, the strong adsorption results in the decomposition of Pro into HCN, ketene and ethylene/acetylene.[262]

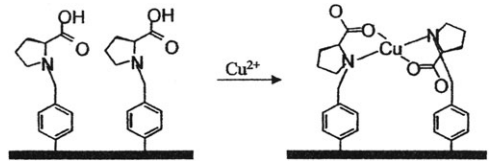
2.4 Synthesis, analytical and biomedical applications of amino acid complexes

A huge number of papers that came out during the period of coverage can be associated with these subjects. However, some of them are beyond the scope of this chapter or are discussed in other parts of this volume, so different metal ion-catalysed routes of effective synthesis to various amino acids/derivatives (*e.g.* refs. 263–267) are not detailed here. Different motivations (*e.g.* analytical or biological aspects) have initiated the work to find effective routes for the synthesis of various metal ion-amino acid complexes. A one-step procedure providing large quantities of several [RuCp*] (Cp* = pentamethylcyclopentadienyl) complexes including some with amino acids, for use in bioorganometallic chemistry, has been developed.[268] 4-Ethynyl-L-phenylalanine is of interest as a potential drug and as a selective inhibitor of tryptophan hydroxylase enzyme. Recently, several new organometallic compounds of this Phe derivative with cobalt, ruthenium and platinum, have been synthesized.[269] In other papers, methods for the synthesis of organophosphorous derivatives of amino acids and their metal complexes as catalysts for various asymmetric hydrogenation reactions,[270] and for preparing Fe(II)-amino acid complexes by hydrolysis of keratin in presence of Fe(II) salt,[271] have been published.

When copper-amino acid complexes are immobilized on supports, as enzyme mimics, efficient and selective catalysts in a large variety of reactions, can be obtained. A procedure for the synthesis of polymer-supported, immobilized Cu(II) complexes with L-Val has been presented. These supported complexes behave as versatile catalysts in the oxidation of various substrates such as benzyl alcohol, cyclohexanol and styrene in presence of *t*-butyl hydroperoxide as the oxidant.[272] In another work, Cu(II)-amino acid complexes have been immobilized in montmorillonite or on silica gel.[273] If one of the two components of a metal complex, either the metal ion or the ligand is immobilized on a solid support, the recognition of the another component might be achieved under certain conditions. Several new results have appeared during the past two years in this subject, *e.g.* selective detection of Cu(II) ions in aqueous solution was achieved by the IR-sensor constructed by surface modification with L(−)-Pro. Scheme 2 shows the schematic diagram of the surface of the immobilized L(−)-Pro phase before and after complexing with Cu(II).[274]

In another work, a copper nanoparticle-plated screen-printed carbon electrode (designated in Scheme 3 as Cu^{II}-$SPE_{100\text{-}nm}$) was developed for determination of native amino acids. The reaction mechanism for the Cu(II)-amino acid complexation at the surface of electrode is shown in Scheme 3.[275]

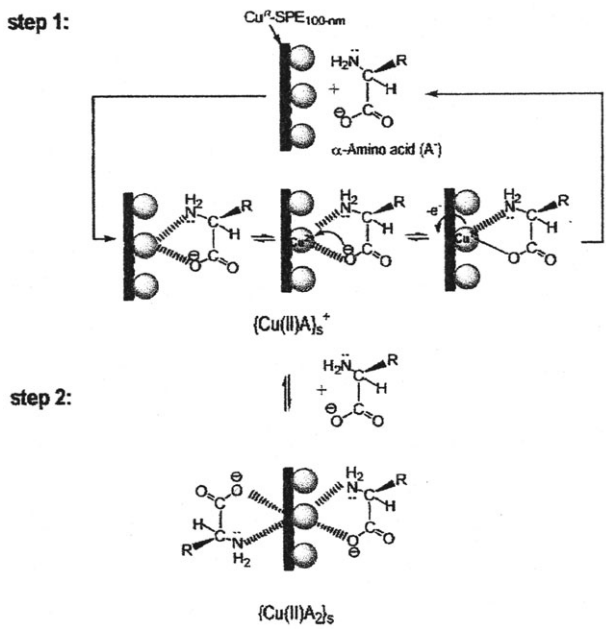
The formation of stable L-tyrosine-aluminium cluster was obtained in a supersonic beam expansion of a laser-ablated L-Tyr and Al target.[276] Several new amino-acid-based adsorbents for removal of different metal ions have been



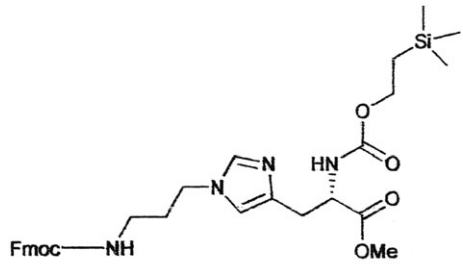
Scheme 2

developed during the past two years, *e.g.* amino acid-modified chitosans for removal of Cu(II) ion,[277] methacryloylamidoglutamic acid (functionalized poly(2-hydroxyethyl methacrylate) beads) for removal of UO_2^{2+},[278] methacryloylamidocysteine for removal of Cd(II).[279] Water-dispersible, tryptophan-protected gold nanoparticles have been successfully prepared by the spontaneous reduction of aqueous chloroaurate ions by this amino acid.[280] Chiral films of CuO were electrochemically deposited onto achiral Au(001) single crystal surfaces from alkaline solutions of Cu(II) complexes of tartaric acids and the amino acids Ala, Val and Gly.[281] Another interesting method by which copper is efficiently removed from its amino acid complexes uses sodium borohydride reducing agent. In this method, the copper is reduced to insoluble Cu(I)-oxide and the free amino acid is released in pure form.[282]

Labelling of biologically active molecules with ^{99m}Tc is a field of intense research, because this metal is one of the most widely employed isotopes for imaging in molecular medicine. In a recent study, a new histidine derivative (**24**) was prepared and following its conjugation to various biomolecules, the obtained bioconjugates were labelled with $[^{99m}Tc(OH_2)_3(CO)_3]^+$.[283] In another work, a new ^{99m}Tc-labelled derivative of L-Tyr as a potential tumour-imaging agent was synthesized.[284]

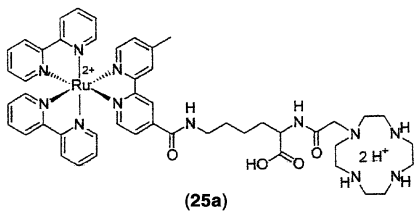


Scheme 3

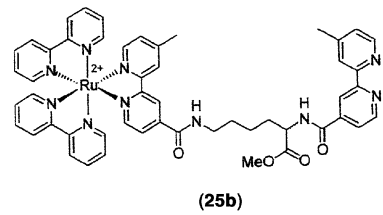


(24)

Luminescent molecules for the recognition of ions in solution generally consist of three subunits: a signal-generating chromophore, a receptor ligand, and a connecting spacer. Luminescent, Cu(II)-binding new chromophore–spacer–receptor conjugates (their structures are shown in (**25**)) have been derived.[285]



(25a)



(25b)

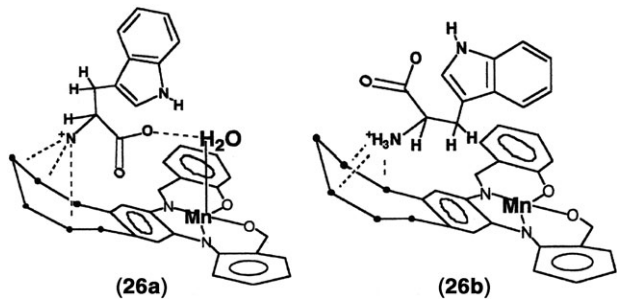
Fluorescent sensors are molecular systems containing a receptor moiety which, conventionally, contains one or more metal centres, and a fluorogenic fragment. The design of fluorescent sensors for anionic groups (including those of amino acids) have been described and the crucial role of the coordinative interaction between the metal centres and the substrate has been demonstrated. As an example, recognition and sensing of amino acid His has been considered in detail.[286] Cu(II) complexes of modified cyclodextrins have been tested as fluorescence sensors for amino acids. It was found that the addition of amino acids to the copper(II) complexes caused increases in the fluorescence. The changes were dependent on the type of amino acids used, and for some amino acids, on the absolute configuration.[287,288]

Chemoselective signalling of phosphorylated Tyr residues was achieved by binding these ligands to Eu(III)- and Tb(III)-aqua complexes. The methods applied during the investigation were luminescence-emission and ^{1}H NMR spectroscopy.[289] Enantioselective effects were observed in electron transfer processes during the luminescence quenching of D- and L-Trp by Eu(III)-L-gluconate as chiral discriminator.[290] Ninhydrin is a well-known, widely used chemical for colorimetric determination of amino acids. According to a recent paper, genipin, a hydrolysate of geniposide from gardenia fruits, can also be used for the same purpose. However, while metal ions such as Cu(II) and Fe(III) affect colorization with ninhydrin, this is not the case with genipin.[291]

Due to the explosive growth in the synthesis and application of enantiomerically pure compounds, analytical methods for the separation and determination of enantiomers have become very important in the past few years. Various separation techniques, including capillary electrophoresis,[292–295] capillary electrochromatography,[295] micro liquid chromatography,[295] HPLC,[296] micellar electrokinetic chromatography,[297] paper chromatography,[298] TLC,[299] affinity chromatography[300] have been used for numerous tasks related to metal ion-amino acid interaction.

Some of the papers during the past two years present different biological models, and/or discuss *in vivo* effects of metal complexes of amino acids/derivatives.[301–311]

Synthetic carriers for the facilitated transport of hydrophilic biomolecules and drugs across cell membranes are rare and the design and synthesis of such molecules are important both for biological and pharmacologycal reasons. Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+} and Cu^{2+} complexes of some crown-functionalized salicylaldimine ligands have been investigated, and their use in the transport of amino acid Trp and serotonin across $CHCl_3$ bulk liquid membranes have been discussed. As illustrations, the energy-minimized structures of the Mn(II)-Trp-^{t}Bu$_4$-salphen-18-cr-6 complexes are presented in (**26a** and **26b**). The energy of (**26a)**, is lower than that of (**26b**).[301]



(26a) (26b)

A potential mechanism for antimalarial action of tetraoxanes was illustrated by reacting the iron complex of cysteinate in the presence of methyl cysteinate with 1,2,4,5-tetraoxane.[302] Structures, chemical properties and *in vitro* insulinomimetic activities of new vanadyl (oxovanadium(IV)) complexes with five imidazole-containing derivatives of amino acids, (*S*)-Gly, (*R*)-Ala, (*S*)- and (*R*)-Leu, have been examined. Among the studied complexes, the one with the tetradentate Gly derivative was found to have the highest *in vitro* activity.[303]

Arginase is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-Arg through a metal-activated hydroxide mechanism to form L-Orn and urea. Arginase inhibition with amino acid sulfonamides,[304] amino acid aldehydes,[305] L-Val and various derivatives of L-Arg[306] have been investigated. Microbial effects of several metal ion-amino acid complexes have also been investigated in several laboratories.[307–309] Complexation with amino acids can play an important role in controlling bioaccumulation.[310,311] Several chelating agents, including Glu, have been used to prepare their complexes with Ni(II) ion for studying the relationship between the chemical form of nickel added to the soil and its uptake and toxicity to barley plants.[312] The relationships between Gln, Glu and γ-amino butyric acid (essential amino acids for brain metabolism and function) in nerve endings under Pb-toxicity conditions have been also studied.[313]

Most biochemistry happens in aqueous solution and the solubility of many stable non-electrolytes (for example solubility of transition metal complexes of amino acids) depends much on the presence of simple salts, such as NaCl, $CaCl_2$, $MgCl_2$. On this subject, anomalous "salting in" effect has been discussed.[314] Solubility investigations, including determination of some thermodynamic parameters, have also been performed in some other laboratories.[315,316] In many cases, the interactions of amino acids with metal ions occur not in simple aqueous solution, but in much more complicated systems. Interaction of Al(III) with amino acids in human blood,[317] and complexation of different metals, such as Al(III), Cu(II), Pb(II), Mn(II), Ni(II), Zn(II) and Cd(II), with Met, Cys and methionine sulfoxide in the presence of humic substances[318] have been studied.

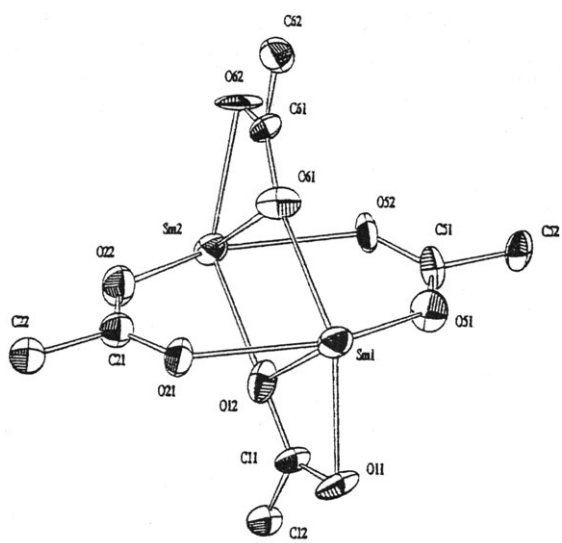
3. Peptide complexes

3.1 Synthesis and structural studies on peptide complexes

A great variety of metal complexes of peptides and related ligands have been prepared and structurally characterized both in solution and in the solid state. These studies were mainly performed by X-ray diffraction analysis of crystalline samples, but various spectroscopic methods (UV-Vis, IR, CD, NMR and EPR) were also extensively used. Moreover, an increasing number of publications are dealing with the application of various forms of mass spectrometry[319–329] and theoretical calculations[330] in the structural elucidation of the most common metal ion peptide complexes.

Organotin(IV) derivatives of amino acids and peptides have received increasing attention in the past two years.[331–334] In addition to the great structural variety of the di- or tri-organotin(IV) complexes of peptides, the significant biological activity of the molecules including antitumor[331] and anti-inflammatory[332,333] activities, have also been justified in these studies. Gold(III) complexes of amide-type ligands including biuret[335] and amides of EDTA and PDTA[336] have been prepared and structurally characterized. It was suggested that the deprotonated amide functions are promising coordination sites for binding of gold(III) compounds. The complexation of samarium(III) ions with small peptides was studied by potentiometric measurements and, in agreement with expectations, only the formation of low stability complexes was detected. As a consequence, the common peptide ligands cannot prevent hydrolysis of lanthanides above pH 6, but the complex $[Sm_2(Gly–Val)_4(H_2O)_8](ClO_4)_6 \cdot 2H_2O$ was isolated at pH 5. In this dinuclear complex two samarium(III) ions, with a coordination number nine, are connected *via* four bridging carboxylate residues. A perspective view of the dimer **(27)** clearly shows that carboxylate bridges are not equivalent; two of them consist of bidentate, but the others include tridentate carboxylate functions.[337]

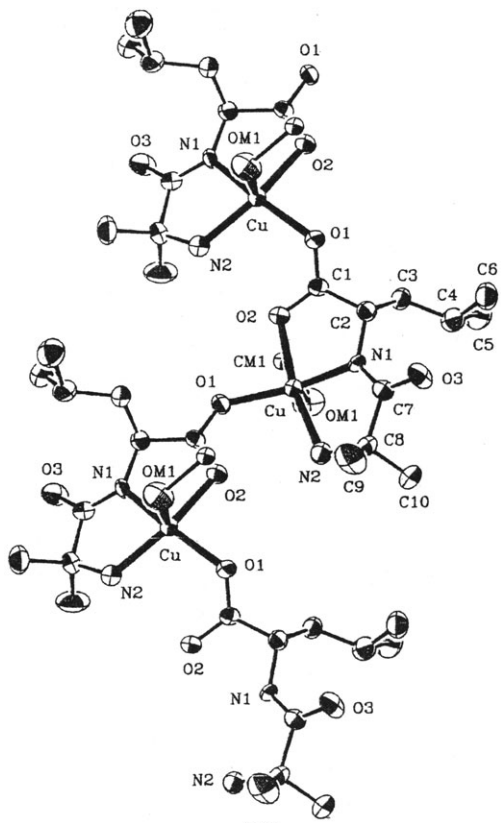
The reaction of vanadate(V) with a series of dipeptides was investigated by UV-Vis and multinuclear (^{51}V, ^{14}N and ^{13}C) NMR spectroscopies. It was found that the complex formation of peptides with vanadium(V) is enhanced by the presence of a functionalized or sterically demanding side chain, *e.g.* by Val–Gln.[338] The alkaline earth metal complexes of N-substituted amino acids and Gly–Gly have been prepared and structurally characterized. Eight-coordinated cations were found in



(27)

the tetrahydrated Sr(II) and Ba(II) complexes of GlyGly and the coordination aggregates were linked *via* an extensive series of H-bonds.[339] Aluminium is a neurotoxic element and its interaction with proteins and peptides received increasing attention. Some of these studies were focused on the kinetic or equilibrium aspects of the interaction and will be discussed in the corresponding subsections. Peptide YY (PYY) and neuropeptide Y (NPY) are members of the pancreatic polypeptide family of neurohormones. Previous studies indicated that these peptides may play a role in the metabolism of aluminium, but most recent studies ruled out direct interaction between peptide and aluminium(III).[340]

The major part of the most recent publications on the metal ion complexes of peptides was devoted to the role of side chain residues in the complex formation reactions. Among them, imidazolyl and thiol functional groups are the best studied and these results are discussed separately in subsection 3.4. Concerning the role of other side chains or their combinations, theoretical studies have been performed for the metal ion selectivity of specific functional groups and the range of metal ions covered cobalt(II), nickel(II), zinc(II), cadmium(II) and mercury(II).[341] The inductive and steric properties of α-carbon methyl groups of α-aminoisobutyric acid (Aib) were evaluated by the structural characterization of copper(II) complexes containing Aib residues. A polymeric structure **(28)** was obtained for the complex $[Cu(H_{-1}L)\text{-}(MeOH)_n]\cdot n$MeOH (LH = H-Aib-L-Leu-OH) indicating the common (NH_2,N^-,COO^-) bonding mode with carboxylato bridges.[342]



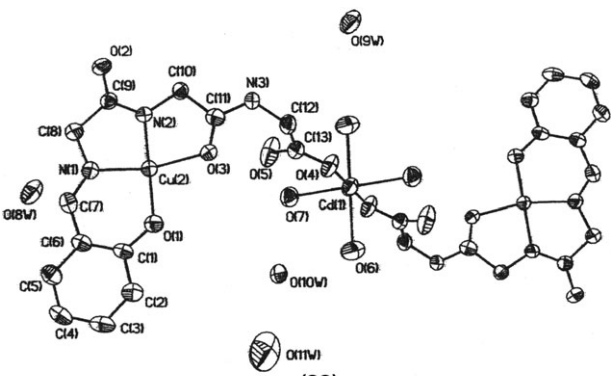
(28)

The interaction of a β-dipeptide (anthranoyl–anthranilic acid) with monovalent metal cations (Li^+, Na^+ and Tl^+) was studied in the solid state in the form of the corresponding salts. The alkaline metal ions show a clear preference for

water molecules over the dipeptide anions, while the coordination of one nitrogen and four oxygen atoms of the anions were suggested in the dinuclear thallium compound.[343]

Biological applications of cyclopeptides and their interactions with metal ions received increasing attention in the past few years. It has been shown that various cyclo-tetrapeptides containing functional groups can be obtained from the corresponding dipeptide esters by template synthesis at nickel(II) and palladium(II) centres. It seems to be a very facile method of preparation in comparison with conventional procedures.[344] Secondary structural preferences of amphipathic cyclopeptides in detergent assemblies and their interactions with metal ions were studied by circular dichroism spectroscopy. The results indicate that cyclo-octapeptides can co-assemble with micellar dodecylphosphocholine (DPC) and are capable of interacting with metal ions.[345] Metal binding properties of the immunosupressant drug cyclosporin A have also been investigated. The results support the possibility that cyclosporin A has ionophoric properties for biologically important essential metal ions.[346] Patellamide D is a cyclic octapeptide possessing a 24-azacrown-8 macrocyclic structure. Its complexation with copper(II) was studied by spectrophotometric, EPR and mass spectroscopic measurements and the formation of mononuclear and chloro-bridged dinuclear species was suggested.[347] Bleomycins are naturally occuring cyclopeptide antibiotics with significant anticancer activity which is attributed to its ability to cleave DNA. It is generally accepted that the meachanism of DNA degradation is both oxygen and metal ion dependent. The complexation of bleomycin and its structural analogues was studied with several metal ions including copper(II),[348] gallium(III),[349] iron(II)[350,351] and cobalt(II)[351] for the identification of the metal binding domains.

Metal ion complexes of Schiff base derivatives of peptides are often considered as promising structural models of metalloenzymes and, on the other hand, they are potential antibacterial and anticancer agents. The heterotrinuclear complexes $[Mg(H_2O)_6][CuL]_2 \cdot 3.5H_2O$ and $[Cd(H_2O)_4][CuL]_2 \cdot 3.5H_2O$ (where H_3L = N-salycylidenglycylglycylglycine) have been prepared and structurally characterized. In the case of the Mg/Cu complex the two metal ions are stacked in well-separated columns, while for the Cd/Cu species **(29)** two symmetric $[CuL]^-$ units coordinate to cadmium(II) *via* carboxylate residues.[352]



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Mixed ligand complexes of peptides are a matter of interest because they are frequently used as functional models of enzymes. The ternary complexes formed in the reaction of copper(II) with 1,10-phenanthroline and simple dipeptides have been structurally characterized and their nuclease activity tested. It was found that the structures of the complexes is significantly influenced by the bulk lateral chains of peptides and it also affects the catalytic behaviour of the complexes.[353] Similar mixed ligand complexes were prepared by the reaction of copper(II) with dipeptides and the

monodentate benzimidazole and creatinine ligands, and the catalase and SOD-like activities of the samples tested.[354] The interaction of zinc(II) and cadmium(II) with the pentapeptide, Asp–Asp–Asn–Lys–Ile, surrounding the thiamin phosphate moiety in the transketolase enzyme, and the ternary Zn(II)/Cd(II)-pentapeptide-HETPP (= 2-[hydroxyethyl]thiamin-pyrophosphate) systems have been studied by NMR spectroscopy. The results provided some structural information on the active site of thiamin-dependent enzymes in solution.[355] Mixed ligand complexes of a chiral diaqua-ytterbium(III) complex with each of the 20 common amino acids and selected dipeptides have been structurally characterized. The axial coordination of the terminal amino functions in a 5-membered chelate with the amide carbonyl oxygen was suggested for all dipeptides within a nine-coordinated mono-capped square antiprismatic coordination environment. Evidence for chelation through side chain functionalities was found only in the case of N-terminal Asp residues.[356] The interaction of plastocyanin with oligopeptides containing four lysyl residues in different positions has been studied by spectroscopic techniques. It was found that the peptides competitively inhibited electron transfer between cytochrome c and plastocyanin. The results show that the binding of oligopeptides to plastocyanin is slightly more efficient when lysines are distributed uniformly within the peptide, whereas structural changes of plastocyanin are more evident when lysines are close to each other.[357]

3.2 Kinetics and reactivity

Only a few publications deal with the determination of kinetic parameters and mechanism of the formation of simple metal ion peptide complexes. On the contrary, the metal ion promoted formation, hydrolysis, oxidation and other reactions of peptides received increasing attention in the past two years.

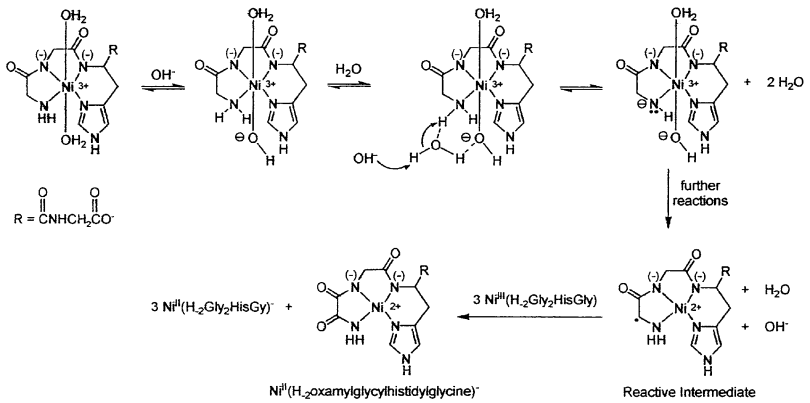
It has already been widely accepted that metal ions played an important role in the formation of amino acids, peptides and related substances under prebiotic conditions. The adsorption of organic substances on the surface of clay minerals is one of the key steps in these reactions. As a consequence, the adsorption of peptides and peptide bond formation on the surface of natural or synthetic montmorillonite and activated alumina has been thoroughly studied by several groups.[358–362] Moreover, the different reaction yields for L- and D-alanine in the salt induced peptide formation reactions point at a stereoselective differentiation in this processes.[363] On the other hand, it is an important observation on the preparative scale that tantalum pentachloride seems to be a very efficient coupling agent for stereohindered amide bond formation.[364]

Metal ion catalysed oxidative degradation and/or transformation of proteins has an outstanding biological significance and peptides are promising models for the understanding these reactions. A sequence specific oxidative degradation of tripeptides by cobalt(III) complexes containing terpyridine ligand has been reported. The cleavage of amide bonds was observed in the case of tripeptides consisting of C-terminal aliphatic amino acids and the selectivity was explained by interligand interactions.[365] Studies on the Fenton-type oxidation (by Fe^{2+}/H_2O_2 system) of model peptides containing Met, Tyr and His residues revealed that metal-bound reactive oxygen species oxidize methionine to methionine-sulfoxide.[366] The oxidation of thiol containing ligands including cysteine and glutathione was studied in the presence of MnO_2. Mn(II) and the disulfides were the products of the reaction in the pH range 4–9, while cysteic or cysteinesulfonic acids were obtained below pH 2.[367] The kinetics of the oxidation of common dipeptides by anodically generated manganese(III) was followed by spectrophotometric measurements. The parameters affecting the rate of oxidation are discussed.[368]

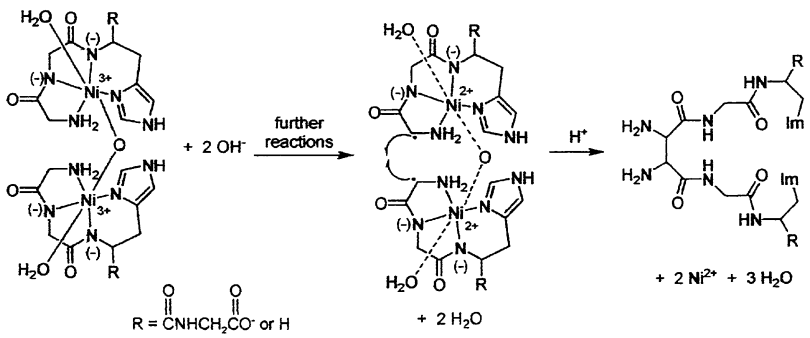
Studies on the oxidation of divalent transition metal ion oligopeptide complexes to trivalent species have been the subject of continuous interest for several years. It has been demonstrated that the oxidation of nickel(II) and cobalt(II) tetraglycine

complexes in borate buffered aqueous solution is strongly accelerated by sulfite ions.[369] In another paper the synergistic effect of nickel(II) and cobalt(II) on the sulfite induced autooxidation of copper(II)-tetraglycine system has been reported. It was found that the addition of trace amounts of nickel(II) or cobalt(II) significantly increases the rate of oxidation and decreases the induction period drastically (from about 3 hours to 0.5 s).[370] A series of papers have been published on the oxidation and decomposition kinetics of copper(III) and nickel(III) complexes of peptides containing histidine or histamine as the third residue.[371–374] In the case of GGHG at least two nickel(III) complexes are reduced to nickel(II) while oxidizing a single peptide ligand. The rate of nickel(III) loss follows first order kinetics at low pH (Scheme 4) and second order at high pH (Scheme 5).[373] In the case of copper, the species [Cu(III)(H_{-2}GGHG)] was generated from the corresponding divalent complex in the reaction with L-ascorbic acid and H_2O_2. The copper(III) complexes decompose to give alkene peptide isomers of GG-α,β-dehydro-HG.[374]

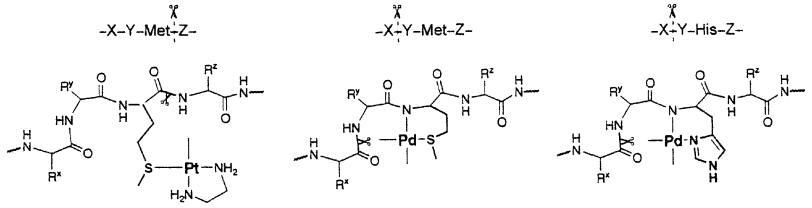
A huge number of papers have been published on the metal ion induced selective cleavage of peptide bonds.[68,375–387] Most of these studies have been performed on the palladium(II) and platinum(II) complexes,[375–379] and histidyl and methionyl residues were identified as the primary binding sites for peptide cleavage. It has already been known from previous studies that the coordinatively unsaturated palladium(II) complexes consistently cleave the second amide bond upstream from histidine and methionine residues; that is the X–Y bond in the sequence segments X–Y–His–Z and X–Y–Met–Z, in which X, Y and Z are any non-



Scheme 4



Scheme 5



Scheme 6

coordinating amino acid residues. On the contrary, $[Pt(en)(H_2O)_2]^{2+}$ was shown to be a new proteolytic reagent (Scheme 6). It was found that the complex hydrolitically cleaves the peptide bonds at the C-terminus of each Met residue; that is the Met–Z bond.[376] The major advantage of this observation that the combined use of platinum(II) and palladium(II) complexes multiply the selectivity in peptide cleavage, *e.g.* in the peptide Ac-AKYGGMAARA the platinum(II) reagent cleaves the M(6)–A(7) peptide bond, while palladium(II) reagent cleaves the G(4)–G(5) bond.[378]

The peptide bonds of Gln(91)–Ser(92) and Ala(94)–Thr(95) were remarkably cleaved by copper(II) complexes anchored to the side chain of the His(93) residue.[380,381] The results support that copper(II)-mediated cleavage of myoglobin is able to proceed at neutral pH and is more selective than the palladium-mediated cleavage. Several papers have been published on the role of zinc(II),[68,382–384] cobalt(III)[385,386] and zirconium(IV)[387] in peptide cleavage. In the case of zinc(II), not only the mild reaction conditions of peptide cleavage have been clarified, but it has also been reported that dipeptides having the serine residue at the C-terminus (X–Ser) were very efficiently hydrolysed in the presence of $ZnCl_2$ at pH 7.0.[383] Cleavage of the peptide bond was reported[385] when carnosine (β-Ala–His) was reacted with $[Co(tren)Cl_2]^+$ (tren = tris(aminoethyl)amine) to give [Co(tren)(histidine)]$^{2+}$ and [Co(tren)(β-alanine)]$^{2+}$. On the other hand, an unusual *trans* cleavage reaction was observed when *trans*-$[Co(3,2,3\text{-tet})Cl_2]Cl$ (3,2,3-tet = *N*′,*N*′-bis(aminopropyl) ethylenediamine) was allowed to react with β-Ala–His in aqueous solution at neutral pH values.[386] The early transition metal ion zirconium(IV) has enhanced Lewis acidity imparted by its stable +4 oxidation state. It was found that the macrocycle (4,13-diaza-18-crown-6) substantially increases the rate of hydrolysis of unactivated amide bonds under physiological conditions.[387]

Peptide complexes of palladium(II) and platinum(II) are frequently used models for the better understanding of the biological activity and transport processes of various platinum containing anticancer drugs. The kinetics of the complex formation reaction between $[Pd(dien)Cl]^+$ and the sulfur containing ligands L-Cysteine and glutathione was studied in the presence of sodium dodecyl sulfate (SDS) micelles. The complex formation was accelerated by the anionic micelles and associative reaction mechanism was suggested.[388] The pH- and time-dependent reaction of the anticancer agent carboplatin with the tripeptides, GGM-OH and Ac-GGM-OH, was studied in comparison with the reactions of cisplatin. The kinetic parameters suggest that the S → N migration of platinum binding could play an important role in accelerating the rate of DNA binding to carboplatin.[389] There is a great interest in the development of new platinum antitumor drugs with increased pharmacological activity and reduced toxicity. The peptide-tethered platinum(II) complexes represent a new category of these complexes and their synthesis and interaction with DNA have been reported recently.[390–392]

3.3 Solution equilibria and speciation in metal ion peptide systems

The equilibrium studies and the determination of the metal ion speciation in solution comprise a major part of metallopeptide chemistry. For the elucidation of the metal

binding sites of the various species, however, a lot of different spectroscopic techniques are used. As a consequence, some of the equilibrium studies have already been cited with the structural aspects of these complexes in subsection 3.1, while those on the peptides of histidine and cysteine will be included in the next paragraph.

pH-Potentiometry is the most common experimental method for the determination of the stability constants of the metal complexes of organic ligands, but various spectroscopic techniques are also increasingly used. Two-dimensional simulation of electron paramagnetic resonance spectra made it possible to identify species present in very low concentration or the occurrence of coordination isomers. The application of this method for the analysis of copper(II)-oligoglycine systems revealed that even diamagnetic dinuclear complexes can be characterized by the EPR measurements.[393]

The evaluation of the equilibrium parameters of peptide complexes makes a significant contribution to the better understanding of the role of side chain residues or modification of the peptide backbone on the complex formation processes of peptide ligands. Nickel(II) and palladium(II) complexes of tripeptides containing β-alanine in all possible locations were studied by potentiometric, UV-Vis and NMR methods. Coordination geometries of the complexes were not affected by the chelate ring size, but the thermodynamic stability of the complexes was significantly influenced by the number and position of β-alanyl residues. The destabilizing effect of the 6-membered chelate from the N-terminal β-Ala residues was observed in all cases. The complex $[MH_{-2}L]$ was detected as the major species with both metal ions, but the stabilities of the different chelate ring sizes followed the order: (5,5,6) $\geq$ (5,6,5) $\geq$ (5,5,5) $>$ (6,5,5) $>$ (5,6,6) $>$ (6,5,6) $>$ (6,6,6) for nickel and (5,6,5) $>$ (5,5,6) $>$ (5,6,6) $>$ (5,5,5) $>$ (6,5,6) $\geq$ (6,5,5) $>$ (6,6,6) for palladium(II). It is clear from these stability orders that a β-alanine present in the internal or C-terminal positions of tripeptides will significantly enhance the palladium binding affinity of the ligands as compared to those of oligoglycines.[394] Thermodynamic and spectroscopic parameters have been reported for the copper(II) complexes of pentapeptides consisting of two dehydro amino acid residues in the amino acid sequence. It was found that the double bond between the α- and β-carbon atoms of amino acids enhances the metal binding affinity of the ligands and this effect is especially pronounced if two dehydro amino acids are inserted into the peptide chain.[395]

Aluminium(III) complexes of Asp–Asp and Asp–Asp–Asp were studied by potentiometric and multinuclear NMR spectroscopic methods. The results demonstrate that the peptides containing Asp residues are effective binding sites for aluminium(III) *via* their extra carboxylate functions in weakly acidic solutions, but the binding strength of this interaction is not sufficient to keep the metal ion in solution above pH 5.[396] Dialkyltin(IV) complexes of various peptides have also been studied by potentiometric and spectroscopic techniques.[397,398] The coordination properties of the simple dipeptide Ala–Gly has been compared to that of the mercapto analogue, MPG = [N-(2-mercaptopropionyl)glycine], and the data confirmed that the thiolate can act as an anchoring group in the diorganotin(IV) induced amide deprotonation. Fig. 4 is used to demonstrate the enhanced metal binding affinity of MPG (a) compared to Gly–His (b), Sal–Gly (c), Asp–Gly (d), Gly–Asp (e), Gly–Gly (f) and Ala–Gly(g).[397]

The interaction of the tripeptide analogue Sal–Gly–Ala was studied with VO(IV), VO_2(V) and Cu(II) ions in aqueous solution. It has been shown that the ligand has almost equally high affinity to bind Cu(II) and VO(IV) *via* the coordination of phenolate-O, two amide-N and carboxylate-O donor atoms. Conversely, the affinity for VO_2^+ binding was significantly lower and no interaction was detected in the pH range 2–12.[399] However, the stability constants of the complexes formed in the reaction of VO_2^+ with simple dipeptides, Ala–Gly and Gly–Ala, have been reported in another study.[400] The metal ion speciation of the quaterner system $H^+/H_2VO_4/H_2O_2$/AlaSer has been determined by potentiometric and ^{51}V NMR measurements.

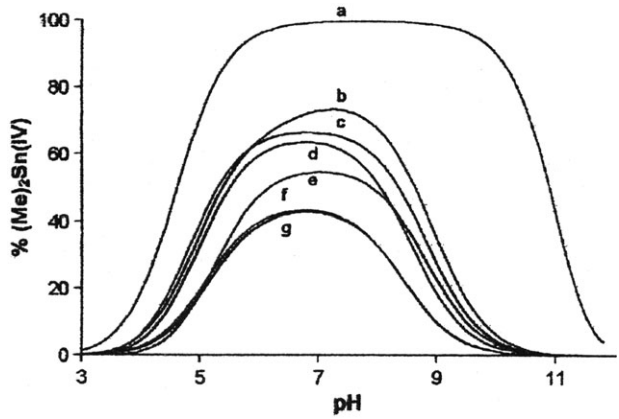


Fig. 4

The results were compared to those of Ala–His and the preference of peroxovanadium species towards aromatic nitrogen or oxygen donating ligands has been demonstrated.[401]

The metal binding affinity of peptide molecules can be finely tuned by the variation of the side chain donor atoms. The weak stabilizing effects of phosphonic oxygen and thioether sulfur donor atoms have been shown in the copper(II) complexes of various derivatives of dipeptides.[402] Coordination of copper(II) to polymers bearing Gly–Gly, Phe or Met in their side chains was studied by potentiometry and viscosimetry. These polymers, which are not polypeptides, have carboxyl and amide group in the side chain and form 2:1 COO:Cu complexes in the lower pH range. The stability of this complex is in the order: PPhe > PMet > PGly–Gly.[403]

The equilibrium studies on the formation of mixed ligand complexes of transition elements with the most common peptides (mainly dipeptides) are the subject of continuous interest.[404–410] The B-ligands in these studies include the chelating hydroxamates,[404] histidine and derivatives,[405,406] diamines and aminopoly-carboxylates,[407,408] various macrocycles[409] and the monodentate imidazole ligands.[410] Potentiometric and NMR studies in the zinc(II)–glutathione–histidine system revealed the existence of ternary species under physiological conditions, and its possible role in the transport processes of zinc(II) is discussed.[405] In the case of a hexaaza-macrocycle the kinetics of the interaction with copper(II) and glycylglycine has also been followed.[409]

3.4 Metal complexes of peptides of histidine and cysteine

The imidazole nitrogen donor atoms of histidyl and sulfur donor atoms of cysteinyl residues are the most common metal binding sites of proteins. As a consequence, the investigation of the metal complexes of ligands consisting of these residues in model or natural peptides received increasing attention in the past two years. The studies in this field generally cover all aspects of the coordination chemistry of the ligands including the clarification of solution equilibria of the systems and the structural characterization of the major species.

Nickel(II) complexes of the tripeptide GHK and its synthetic analogues were studied by potentiometric and various spectroscopic techniques in solution. In agreement with earlier findings on the corresponding copper(II) complexes the governing role of histidyl residue in the complexation with nickel(II) has been concluded. The presence of 3N-coordinated octahedral species was suggested at physiological pH, followed by the formation of a tetrameric square planar complex in alkaline solution. No involvement of lysyl residues in metal in coordination was

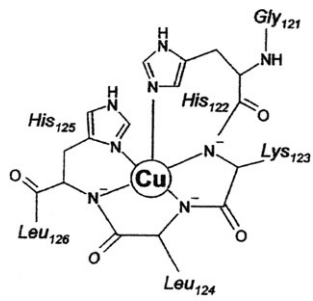
found.[411] Copper(II) complexes of dipeptides containing N-terminal His residues (His–Gly and His–Ala) have been prepared and structurally characterized. The formation of stable bis(ligand) complexes was suggested *via* the exclusive coordination of histidyl residues.[412] The copper(II)-carcinine (β-alanylhistamine) system has been reinvestigated by "two-dimensional" EPR simulation and NMR relaxation studies. In equimolar solution and at pH 7, the formation of oligomerized species was suggested with (NH_2,N_{im})-coordination mode. However, at higher pH values or in the presence of excess of ligand both tridentate and monodentate coordination modes of the ligand in various bis or tetrakis complexes were detected. The SOD-like activity of the system has also been investigated and the complex [$CuH_{-1}L$] was found to be highly active.[413] Copper(II) complexes of the cyclic tetrapeptide, cyclo-(HGHK), have been studied by potentiometric and spectroscopic methods and the results were compared to those of the linear counterparts. The sequential formation of 1N-, 2N-, 3N- and 4N-coordinated complexes involving the equatorial binding of imidazole and amide nitrogen donors was reported. It has also been demonstrated that the copper binding affinity of the cyclopeptide is much lower than that of the terminally free, linear tetrapeptide, but it is comparable to that of the terminally blocked ligand, Ac-HGHK.[414] Some other studies on the metal complexes of simple histidine-containing peptides covered the application of these complexes in various biochemical processes. A study has been reported on the minor groove recognition of A/T-rich DNA sites by nickel(II) complexes of L- and D-Arg–Gly–His.[415] A combined UV-Vis, CD and ESI-MS spectroscopic study has been performed for the characterization of the copper(II) complexes of β-cyclodextrin functionalized by carnosine. A new metal-assisted self-assembled system of bifunctionalized β-cyclodextrins has been shown to exist.[416]

Histidine-containing small peptides are frequently used to mimic the structure and catalytic activity of the CuZn-SOD enzyme.[417–420] Copper(II) and zinc(II) complexes of the peptides related to the copper and zinc binding sites of the enzyme, HVH and HVGD both in free and terminally blocked forms, have been studied by potentiometric and spectroscopic measurements.[417,418] It was found that both peptides have high metal binding affinity, but the tripeptide containing two histidyl residues is a stronger chelating agent than the tetrapeptide. It has also been demonstrated that both copper(II)-HVH and Ac-HVH-NH_2 systems exhibit catalytic activity towards the dismutation of superoxide anion, but the saturated coordination sphere of the metal ion results in relatively low reactivity as compared to the native enzyme.[418] The superoxide dismutase activity of β-cyclodextrin functionalized by carnosine has also been tested. The results reveal that these peptide conjugates are effective SOD-like compounds and they work also as scavangers towards hydroxyl radicals.[419] N-Terminal protected tri- and tetra-peptides containing two or three histidyl residues are also promising structural models of superoxide dismutase or other multiimidazole centered enzymes. The copper(II) complexes of Ac-HGH-OH, Ac-HGH-NHMe, Ac-HHGH-OH and Ac-HHGH-NHMe have been studied by combined potentiometric and spectroscopic techniques. The formation of relatively stable macrochelates was detected in slightly acidic solution, but the (N_{im},N^-,N_{im})-coordinated complexes were the predominant species in all systems at physiological pH. Further increase of pH resulted in the formation of 4N-complexes in the form of (7,5,6)-membered chelate rings.[420]

Metal complexes of the tetra- to hexa-peptide fragments of histones have also been the subject of interest.[421,422] The interaction of zinc(II) with the terminally blocked hexapeptide models, Ac-TESHHK-NH_2, Ac-TASHHK-NH_2 and Ac-TEAHHK-NH_2, was followed by potentiometric and ^{1}H NMR measurements. The results support that the ESHH sequence is a potential binding site for zinc(II) ions, but only imidazole-N donor atoms were suggested as metal binding sites and the possibility of amide coordination was excluded.[421] Previous studies on the copper(II) and nickel(II) complexes of the same hexapeptides came to the conclusion that the metal ions promote hydrolytic cleavage at Gly–Ser amide bond. The most recent studies on the

synthetic tetrapeptides SHHK and SAHK revealed that the high thermodynamic stability of the complexes is the driving force of hydrolytic reactions.[422]

SPARC (secreted protein, acidic and rich in cysteine) is a calcium binding protein, but its interaction with copper(II) may also be biologically important. Copper(II) complexes of the terminally protected short peptide fragments, SPARC(122–126) (= Ac-HKLHL-NH_2), SPARC(121–126) (= Ac-GHKLHL-NH_2) and SPARC(120–126) (= Ac-KGHKLHL-NH_2) were investigated by potentiometric and spectroscopic measurements. The coordination chemistry of the three ligands is very similar: His residues are the primary metal binding sites, followed by the deprotonation and coordination of three amide nitrogen atoms **(30)** in alkaline solutions.[423]



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The interaction of copper(II) with the terminally free pentadecapeptide fragment of SPRAC(114–128) (TLEGTKKGHKLHLDY) has also been studied. The results support that the ligand has two different binding sites for copper(II) ions: the terminal amino group and H(9) and H(12) residues, but the formation of dinuclear complexes was suggested only in the case of excess of metal ions.[424]

Cap43 is a nickel(II) induced protein having a monohistidine motif consisting of 10 amino acids (TRSRSHTSEG) repeated three times in the C-terminus. The nickel(II) binding ability of the 20- and 30-mer peptide fragments containing 2 and 3 separated histidyl residues was studied by potentiometric and spectroscopic measurements. The formation of mono-, di- and tri-nuclear complexes was suggested in octahedral species below pH 9 and in square planar complexes at high pH values.[425]

The possible role of copper(II) ions in the development of human prion diseases promoted a great number of studies on the metal binding ability of prion proteins and its peptide fragments. The results of previous studies have been reviewed in the last two years.[4–6] The more recent studies were performed to understand the metal binding affinity of the protein as a whole[426–429] and also of the various peptide segments[430–442] containing the natural sequences of the protein. The results reported on the role of copper(II) or other metal ions in the pathology of the disease are still very contradictory. Some publications on the interaction of prion protein with copper(II) provide further support on the basic role of the metal ion in the course of the disease. For example, it has been demonstrated that the treatment of mice with the well-known copper(II) chelator D-penicillamine delayed the onset of prion disease and reduced the copper level in brain.[426] On the contrary, another paper demonstrated that the copper(II) binding to prion protein may inhibit prion disease propagation.[427] It was also suggested that prion infection modulates copper content at cellular level and that modification of copper homeostasis plays a dominant role in the neuropathology of the disease.[428] Doppel is a protein with 26% sequence identity with prion protein, but lacks the octarepeat region formerly implicated as the major copper-binding domain. Contrary to expectations, it was found that doppel protein binds copper efficiently justifying

that further studies are required for the elucidation of the metal binding domains of prion proteins.[429]

Most studies on the metal binding ability of peptide fragments of prion proteins were performed with the octarepeat domain of human prion protein (HuPrP).[430–438] This domain is built up from four histidine-containing octapeptides with the repeated sequence, PHGGGWGQ, and consists of the 60–91 amino acids of HuPrP. Computational studies on the interaction of copper(II) with the simplest analogues of the octarepeat, HGGG and HG peptides, revealed that, in agreement with the earlier experimental findings, these sequences are efficient metal binding ligands.[430] Copper(II) complexes of four synthetic tetrapeptides (Ac-HGGG, Ac-GHGG, Ac-GGHG and Ac-GGGH) bearing a single histidyl residue in all possible positions have been studied by potentiometric and spectroscopic measurements. Imidazole-N donor atoms of His residues were the primary metal binding sites in all cases, followed by the successive deprotonation of three amide functions in ($3N^-$,N_{im})-coordination, except Ac-GHGG where only 3N complexes were formed, with the involvement of two amide functions.[431] The basic coordination chemistry of the monomeric octarepeat has been published earlier and reviewed recently.[4] Moreover, copper(II) complexes of some derivatives of the monomeric octarepeat and the dimeric and tetrameric fragments were studied in the last two years. Two peptides containing Ala_3 and Lys_3 units instead of Gly_3 of the octapeptide have been synthesized and their metal binding affinity was compared to that of the natural sequence. It was found that the replacement of Gly_3 with Ala_3 or Lys_3 does not change the basic coordination mode at physiological pH (namely the [N_{im},N^-,N^-,O]-coordination in [7,5,5]-membered chelates), but significantly reduces the thermodynamic stability of the corresponding complexes.[432] The metal binding capacity of the octarepeat and its Ala_3 counterpart is compared in Fig. 5.

The copper binding affinity of the monomeric, dimeric and tetrameric octarepeats were compared in another study. The stability constants of the complexes clearly show that the longer fragments are much more effective ligands than the simple octapeptide. The stability increase could derive from the fact that coordination of the first metal ion involves a multi-imidazole environment.[433] Copper(II) complexes of the monomeric octarepeat have been compared to those of another octapeptide fragments of HuPrP both in the linear and cyclic forms, Ac-GWGQPHGG-NH_2 and c(GWGQPHGG). The results suggest a similar copper(II) coordination mode for the linear peptide in the HGG-domain, while the coordination pattern of the

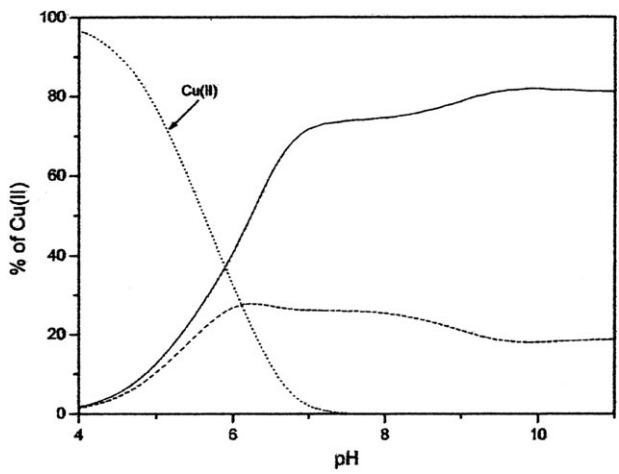


Fig. 5

cyclic analogue was significantly different at physiological pH.[434] The tandem repeat region of chicken prion protein (ChPrP) consists of hexapeptide units (HNPGYP). The major difference between the octa- and hexa-peptide domains of HuPrP and ChPrP is that the amino acids present in the third position after His residue are different; Gly is replaced by Pro which is considered as a break-point for peptide amide coordination. As a consequence, similar to the octarepeat, the metal ions anchor at the imidazole nitrogen and then bind to the adjacent amide nitrogen, but the coordination of the second peptide nitrogen is protected by the Pro residue, thus the third donor atom comes from the phenolate of Tyr residue. This type of metal binding, however, has much lower stability than that of the human octarepeat.[435] Copper(II) complexes of several fragments of human octarepeat and avian hexarepeat domains were compared also with CD spectroscopic measurements. This study also proved the different coordination environment and geometry of the copper(II) complexes of human and avian peptide fragments. It has also been suggested that copper(II) binds cooperatively to the octarepeat domain causing the unstructured N-terminus to fold up in a specific manner. On the other hand, the metal binding was found to be specific for copper(II), and manganese(II) binding to the octarepeat was not observed.[436] Previous structural studies of the mammalian prion protein suggested that the N-terminal domain, consisting the octarepeat, is flexibly disordered. However, in a more recent study it has been concluded that this domain constitutes a pH-dependent folding and aggregation site and, as a consequence, the binding of copper(II) induces a conformational transition that presumably modulates PrP aggregation.[437] It has also been suggested that cell membrane surface can interact with the N-terminal tail of the protein and micellar environments can induce structuring of this domain.[438]

The 21-mer human prion protein fragment HuPrP(106–126) also contains a histidyl residue, KTNMKHMAGAAAAGAVVGGLG, and it is a highly fibrillogenic peptide, resistant to proteinases, toxic to neurons and often considered as the simplest model for the neurotoxic action of the protein. In agreement with this expectation, the most recent study on the neurotoxic properties of HuPrP(106–126) revealed the generation of hydrogen-peroxide, but only in the presence of copper(II).[439] The generation of hydrogen-peroxide from the mutant forms of prion fragment HuPrP(121–231) was also observed in the reaction with iron(II) *via* Fenton reactions.[440] The copper(II) complex of a shorter and more soluble, but N-terminally free fragment, HuPrP(106–113) KTNMKHMA-NH_2, has been studied by potentiometric and spectroscopic techniques. The results indicate that terminal amino, imidazole and amide nitrogens are the metal binding sites, while the side chains of Met and Lys residues do not interact with copper(II).[441]

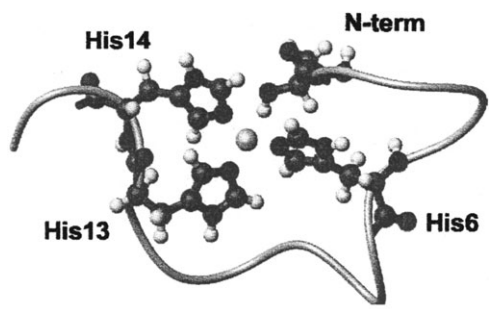
To verify the tendency of copper(II) to interact with the C-terminal structured region of human prion protein, the N-terminally blocked tetradecapeptide fragment, HuPrP(180–193) VNITKQHTVTTT, has been synthesized with both free and blocked C-terminus. The results support the binding of copper(II) at H(187) residue and the similarities in the spectroscopic parameters suggest a common metal binding motif in the different regions of the prion protein.[442]

The histidine-rich amyloid-β peptide (Aβ) is the principal constituent of plaques associated with Alzheimer's disease and is thought to be responsible for the neurotoxicity. A central, unresolved question in the pathophysiology of the disease relates to the role of metal ions in plaque formation and neurodegeneration. As a consequence, a great number of studies have been performed on the metal complexes, especially on the copper(II) complexes, of Aβ peptide.[443–449] Some of these studies suggest that trace metal contamination initiates the apparent auto-aggregation, amyloidosis and oligomerization of Aβ peptides.[443–446] EPR studies on the interaction of copper(II) with the soluble and fibrillar Aβ revealed the presence of mononuclear metal binding site, which does not change during organization of Aβ monomers into fibrils.[446] On the other hand, it has been reported

in another study that the divalent metal ions have only marginal role in the precipitation reactions.[447] Studies on the interaction of Aβ with membrane lipids revealed that pH, the presence of metal ions and cholesterol significantly influence this interaction.[448] The metal ion dependence of the British amyloid peptide (ABri) has been first evaluated. It was found that aluminium(III) and iron(III) increase significantly both the number and size of the fibrillar amyloid deposits, while incubation of zinc(II) or copper(II) precipitated the peptide, but did not result in the formation of amyloid fibrils.[449]

More and more publications support the general view that the formation of reactive oxygen species maybe involved in the pathophysiology of neurodegenerative disorders.[450–453] It is clear from these studies that the presence of metal ions and the methionyl residue in the C-terminal domain are responsible for the redox activity,[450,451] but the role of dityrosine crosslinking have also been demonstrated.[452] The copper(II) catalyzed oxidation of N-terminal human and mouse peptide fragments was studied in the presence of H_2O_2 and the histidyl residues were identified as the primary oxidation targets, but oxidative decarboxylation of aspartic acid and peptide bond cleavage were also suggested.[453]

A series of various spectroscopic and potentiometric measurements have been performed for the clarification of the interaction of copper(II) with the different peptide fragments of Aβ.[454–459] Proton NMR measurements on the interaction of copper(II) with Aβ(1–28), DAEFRHDSGYEVHHQKLVFFAEDVGSNK, indicates that amino terminus and all histidines are responsible for metal binding **(31)**, but Tyr(10) is not.[454]



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A combined potentiometric and spectroscopic study on the copper(II) complexes of Aβ(1–16), and Aβ(1–28) fragments of both human and mouse peptides came to the same conclusion on the role of Tyr residues, but in addition to amino and histidyl nitrogens, the binding of amide nitrogens was also suggested in alkaline solution.[455] The copper(II) binding features of the terminally blocked APP(145–155), Ac-ETHLHWHTVAK-NH_2 and APP(145–157), Ac-ETHLHWHTVAKET-NH_2, fragments of the amyloid precursor protein (APP) were studied by NMR, UV-Vis, CD and EPR spectroscopic methods. Similarly to previous observations the imidazole rings of all three histidyl residues (147, 149 and 151) are involved in metal ion coordination, but the coordination sphere of the metal ion is completed by two amide functions, from L(148) and H(149), at neutral pH **(32)**.[456]

Copper(II) complexes of a heptapeptide (EFRHDSG) corresponding to Aβ(3–9) were studied by Raman spectroscopy and H(6), E(3), D(7) and terminal amino groups were suggested as the metal binding sites.[457]

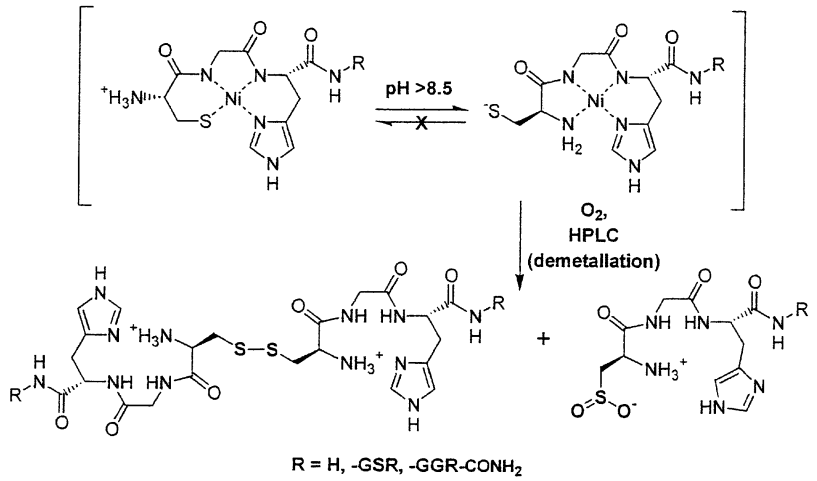
(32)

The role of zinc(II) in neurodegenerative disorders also received increasing interest. The zinc(II) binding properties of Aβ(1–16) were studied by ESI-MS technique. The peptide was shown to form a 1:1 complex with zinc(II) in a hairpin-like conformation, in which R(5)–H(6) and H(13)–H(14) are the binding sites.[458] The interaction of zinc(II) with rat Aβ(1–28) was followed by NMR spectroscopy. It was found that affinity of zinc(II) towards rat Aβ(1–28) is lower than that for human peptide and R(13), H(6) and H(14) were identified as the zinc binding sites.[459]

In addition to the metal complexes of the widely studied prion proteins and amyloid peptides, the metal binding affinity of His residues was reported in many other proteins and their fragments.[460–468] Gonadotropin-releasing hormone (GnRH) is a decapeptide (pEHWSYGLRPG) playing essential role in the neuroendocrine control of the reproductive processes. Because of the low solubility of its metal complexes the interaction of GnRH with nickel(II) was studied in DMSO. The metal ion was found to coordinate with four nitrogen atoms from H, W, S and Y residues including a well-organized arrangements of aromatic side chains and a rigid backbone structure.[460] Copper(II) binding of the peptides P24 and P34, containing 24 and 34 amino acids, were studied by a variety of spectroscopic techniques. The peptide sequences were selected to match a portion of the highly conserved histidine-rich region of the enzyme lysyl oxidase. The binding of one copper(II) was suggested by the involvement of three histidyl-N and one carboxylate-O donor atoms in metal ion coordination.[461] The metal binding affinity of the 18-amino acid peptide fragment of bindin, a membrane associated protein, has been studied by mass spectrometry. High affinity for copper(II) binding has been established, whereas zinc(II)-affinity was found to be comparable to other metal ions including magnesium(II), calcium(II), manganese(II) and lanthanum(III).[462] Metal ion induced folding of peptides with the involvement of $M–N_{im}$ binding is one of the central issues in metallopeptide chemistry.[463–467] The octapeptide (Ac-HAAHHELH-NH_2) was reported to react with two equivalents of $[Pd(en)]^{2+}$ to form a kinetically stable intermediate **(33)** in which two 19-membered metallocyclic rings stabilize two peptide turns. In several hours it was transformed to the thermodynamically more stable species **(34)** containing 22-memberd rings.[466]

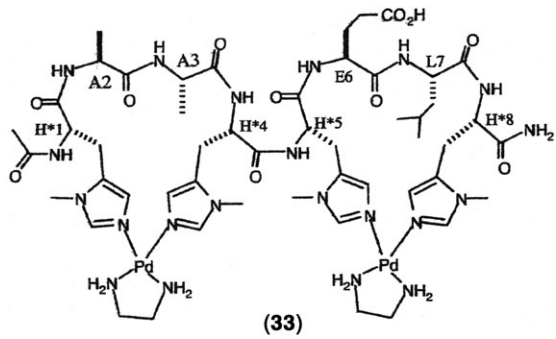
In the case of a linear decapeptide (HGASYQDLGH), nickel(II) ions were found to induce cyclization of the peptide and it was concluded that even insertion of only one His residue at each end of the short bioactive peptide seems to cause a tighter and, hence, more predictable folding.[468]

The ''X–Y–His-'' sequence of amino acids corresponds to the N-terminal domain of human serum albumin and it has been well-known for its high copper and nickel binding affinity. A more recent study on the nickel(II) complexes of Cys–X–His ligands revealed that the presence of thiol at the N-terminus further enhances the metal binding capacity and increases the versatility of the coordination chemistry of the peptides, including a pH- and oxygen-dependent behavior as shown by Scheme 7. The oxidation reaction included the formation of a nickel(III) intermediate.[469] The nickel(II) complex of another tripeptide containing cysteine at both termini, Cys–Gly–Cys, has also been studied. The formation of square planar mono- and dinuclear species were proposed and they can be considered as structural models of the catalytic site of acetyl coenzyme A synthase.[470]



Scheme 7

The peptides containing both histidyl and cysteinyl residues are generally used to mimic the metal binding ability of zinc finger proteins and related substances. The studies on the zinc(II) complexes of these peptide fragments provided further insight into the thermodynamic stability and coordination geometry of various types of zinc



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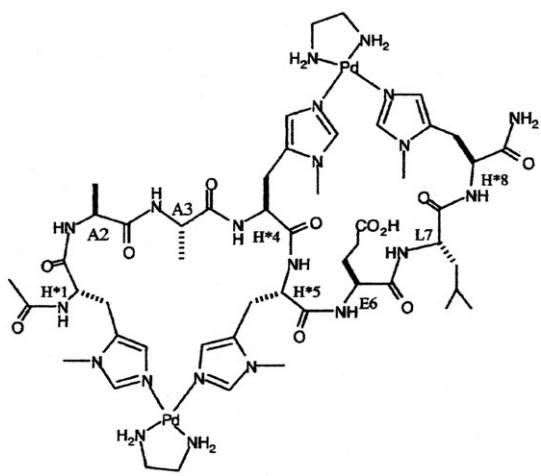

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finger domains.[471–478] Moreover, it has been reported that mutant peptides, such as zf(CCHG) and zf(GCHH) exhibit catalytic activity in hydrolytic reactions. The catalytic activity was attributed to the presence of free coordination sites in the complexes of mutant peptides in contrast with the wild-type sequence, zf(CCHH), which is able to saturate the coordination sphere of the metal ions.[479] The origin of metal ion selectivity is one of the major questions in the coordination chemistry of zinc finger models. On the basis of spectroscopic measurements and theoretical calculations it has been concluded that ligand field stabilization energies provide an important, but incomplete description of metal ion selectivity.[480] In contradiction with the high metal ion selectivity of zinc finger models, a number of reports indicate that several metal ions can replace zinc(II) in zinc finger proteins and impair or alter their functions. Zinc(II), cadmium(II) and lead(II) binding of TFIIIA has been studied and it was found that both cadmium(II) and lead(II) disrupt the proper binding of TFIIIA to its cognate DNA sequence.[481]

The model peptides of Wilson ATPase are rich in cysteine residues and they can also contain histidine. Spectroscopic studies indicated that the thiol functions of these peptides act mainly as bridging ligands for copper(I) binding and the formation of various oligomeric species was detected.[482]

Most of the publications on the metal complexes of peptides containing thiol functions as the primary ligating sites were focused on the coordination chemistry of glutathione (γ-Glu–Cys–Gly) and its derivatives[483–495] or on the model peptides of metallothioneins.[496,497] Zinc(II) complexes of glutathione and related substances have been prepared in solid state and structurally characterized. The formation of sulfur bridged polynuclear species was suggested, although solution equilibrium studies indicated the presence of mononuclear species in some cases.[483] Glutathione is one of the most likely intracellular reductants of chromium(VI) compounds and believed to be involved in the chromium(VI) induced genotoxicity and carcinogenecity. As a consequence, the interaction of Cr(VI) compounds with glutathione is a matter of increasing interest.[484,485] In contrast with the previous expectations, studies on the reduction of Cr(VI) with glutathione suggest that Cr(V)-glutathione complexes are only short-lived intermediates and the Cr(V) species are mainly bonded by carbohydrate ligands.[484] In the most recent study on the interaction of Cr(VI) with glutathione and two model thiol compounds, Cr(VI)-complexes $[CrO_3(SR)]^-$ with tetrahedral geometry have been prepared and there was no evidence for the formation of relatively stable Cr(IV) intermediates.[485] Mercury(II)-bis-thiolate complexes of glutathione and its amino acid and dipeptide derivatives have been studied by mass spectrometry. The different fragmentation processes of the various complexes were interpreted in the light of metabolism and toxicity of mercury under biological conditions.[486] Solution equilibria of the nickel(II)-glutathione system have been reinvestigated and the formation of a series of mono- and di-nuclear species was suggested. The predominance of octahedral coordination geometry was proposed at low pH values, while square planar complexes were favoured by increasing pH *via* sulfur coordination. It has also been reported that complexation with nickel(II) accelerates air oxidation of glutathione in alkaline solution.[487] The complex formation between nitrosoglutathione and zinc(II), cadmium(II) and nickel(II) was studied by potentiometric and spectroscopic techniques. The stability of the ligand was found to increase in the presence of zinc(II) or cadmium(II) and decrease with nickel(II).[488] The reaction of glutathione with aquacobalamin has also been studied and a rapid and irreversible formation of glutathionyl-cobalamin was detected. It has also been suggested that the reaction played an important role in vitamin B_{12}-dependent processes.[489]

The investigations on the complexes of glutathione also covered interactions with arsenic and antimony. The arsenic(III)-cysteine and glutathione systems have been studied by potentiometric and NMR measurements and only binding of sulfur atoms was suggested.[490] Trypanathione is a conjugate of the tripeptide glutathione

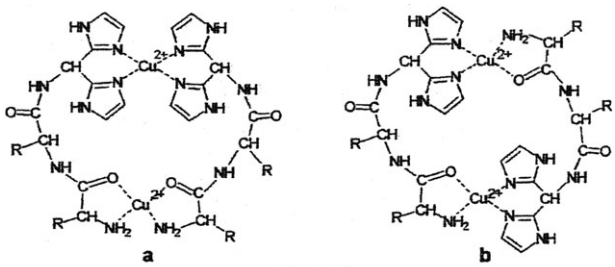
and the polyamine spermidine. It has been shown that the ligand can easily reduce antimony(V) to antimony(III) and the latter can form a stable complex *via* the coordination of thiolates of cysteinyl residues. The results may help to understand the antileishmanial activity of Sb(V) drugs.[491] Phytochelatins are structurally related to glutathione and considered as heavy metal inactivating peptides distributed widely in plants. The cadmium(II) binding ability of (γ-Glu–Cys)$_2$–Gly, a short phytochelatin, has been studied by potentiometric and spectroscopic methods in solution. The results indicate the outstanding cadmium(II) binding affinity of the peptide.[492]

Various sulfur containing ligands play an important role in the transport processes of platinum anticancer agents and, as a consequence, the reactions between palladium(II) and/or platinum(II) complexes and glutathione, in both reduced and the oxidized forms, are frequently studied.[493–495] In addition to the high affinity of these ligands for palladium and platinum binding, it has also been reported that both metal ions can induce reductive cleavage of the disulfide bonds of oxidized glutathione.[494,495]

Complex formation reactions of peptides containing disulfide bridges were studied with copper(II),[498] vanadium(IV)[499] and also with calcium(II), erbium(III) and terbium(III).[500] The specific conformation of the peptide molecules stabilized by the disulfide moieties generally resulted in the enhancement of the thermodynamic stability of the complexes, but there was no evidence for the formation of a direct M–disulfide bonds.

3.5 Synthetic, analytical and biomedical applications of metal complexes of peptides and peptide conjugates

The construction of peptide based supramolecules, capable of performing novel functions represents a great challenge and rapidly growing area in the field of metallopeptide chemistry. These derivatives generally contain another strongly coordinating agent linked to the natural biomolecules. Conformational and coordination properties of a peptide containing a novel chelating agent, α,α-bis(2-pyridyl)glycine (2Dpy), have been studied in solution and in solid state. The tripeptide, Z-Aib-Dpy-Aib-OMe, was able to self-assemble in the presence of copper(II) ions, giving rise to an octahedral complex, with 2:1 peptide:copper(II) stoichiometry.[501] A great number of derivatives of amino acids and peptides containing the bis(imidazol-2-yl)methylamine, BIMA, chelating agent linked to the C-termini of the bioligands *via* an amide bond were synthesized in the last few years and their complexation studied by several transition elements. Some of the results obtained for the simple amino acid and histidine derivatives of BIMA have already been reviewed,[7] while the data for the derivatives of aspartic and glutamic acids and dipeptides have been reported recently.[502–504] The imidazole nitrogen donor atoms were described as the primary metal binding sites in all cases, followed by the formation of ligand bridged dinuclear complexes in equimolar solutions. Spectroscopic measurements indicated that the symmetrical arrangement of donor sites **(35a)** is favoured over the asymmetrical one **(35b)**. Deprotonation and coordination of the amide groups of dipeptides took place in slightly alkaline solutions resulting in the [MH$_{-2}$L] complexes of copper(II) and nickel(II) **(36)**. Metal ion coordination of one of the imidazole nitrogen donor atoms promoted the ionization of N(1)H groups in copper(II) complexes, [CuH$_{-3}$L] (**36**), and in the presence of metal ion excess it resulted in the formation of trinuclear complexes **(37)**.[185]



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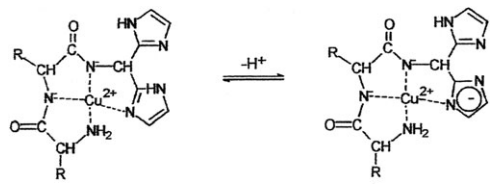

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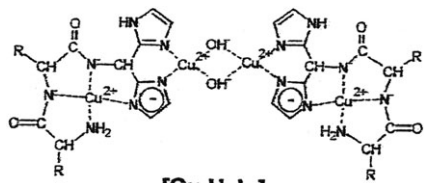

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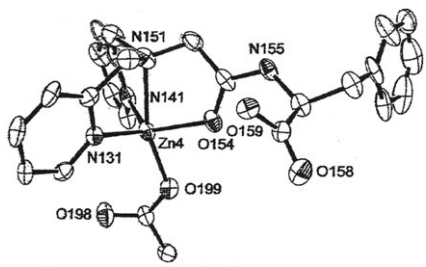
The complex formation processes of these peptide derivatives were especially complicated when histidyl residues were present in the peptides. In the case of His-Phe-BIMA, the histidyl residue at the N-terminus enhanced the stability of the ligand bridged dinuclear complexes, while the $[NH_2,N^-,N_{im}]$ tridentate coordination mode was favoured for Phe–His-BIMA.[504] Dipicolyl-glycylphenylalanine represents another group of metal binding ligands based upon peptides. This molecule easily forms a stable mononuclear adduct with zinc(II) **(38)** and it has also been demonstrated that its flexible amide bond can easily switch between neutral oxygen and anionic nitrogen coordination.[505]



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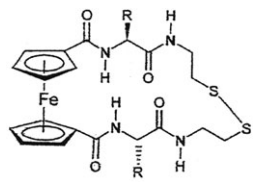
The development of a novel nickel(II) chelator peptide built up from octaarginine bearing nitrilotriacetic acid has also been reported and successfully applied for

peptide-mediated protein delivery into living cells.[506] The zinc(II) complex of the N-substituted Gly–Gly, with 4-amino-1,6-dihydro-1-methyl-5-nitroso-6-oxopyrimidin-2-yl as a substituent, has been structurally characterized.[507] Peptide bond modification *via* N-hydroxylation is an effective strategy to increase the metal ion binding affinity and selectivity of peptides. Novel, linear and cyclic *N*,*N*′ dihydroxy-peptides were synthesized and their iron(III) binding affinity studied.[508] The conjugation of peptide nucleic acids (PNA) with metal binding ligands is also a new strategy among the various bioconjugates. It has been demonstrated that DNA affinity of a bis-picolylamine-PNA conjugate is strongly dependent on the presence of zinc(II) and nickel(II) and to a lesser extent copper(II).[509] The conjugates obtained by the coupling reactions of macrocyclic ligands with peptides have many different biological applications.[510–513] Among others, they can be used as MRI contrast agents,[510] enzyme mimics,[511] or metalloprotein models as mimochromes.[512] "Pegilation" [PEG—poly(ethylenglycol methyl ether)] is a commonly used conjugation to increase the solubility of peptides. A new transition metal mediated living radical polymerization has been successfully developed for the synthesis of this type of peptide or protein conjugate.[514]

The modification of biological molecules with organometallic compounds has attracted much attention in recent years. Ferrocenoyl-peptides and other metallocene derivatives are commonly used redox probes, because their electrochemical properties are able to respond to the structural changes that take place upon substrate binding. A great number of papers have been published on the synthesis, structural characterization and various applications of these metallocene-peptide conjugates.[515–523] The metal ions in these studies include iron,[515–519] cobalt,[515] molybdenum[520] and ruthenium.[521–523] The ferrocene-peptide macocycles represent a new class of these compounds **(39)** and its structure in solid state and electrochemical behaviour in solution have also been determined.[517] A new ferrocenoyl tetrapeptide, Fc–Gly–Gly–Tyr–Arg–OH, has been synthesized in another study and it was found to act as an effective and competitive inhibitor to papain.[518]



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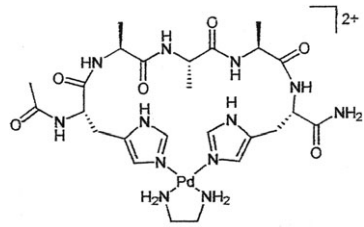
A new and rapidly growing area in the coordination chemistry of peptides is the synthesis and structural characterization of metallo-cyclopeptides.[524–531] In most cases, the side chains or the C- and N-termini of the peptides are functionalized by N- or O-donor ligands and linked by various metal complexes. Palladium(II) complexes are among the most common linkers[524–526] as represented by the macrocycle **(40)** formed in the reaction of $[Pd(en)]^{2+}$ with the pentapeptide HAAAH and in this case the side chain imidazoles of His residues are the linking moieties.[524] Cations of oxomolybdenum(VI)[524,527,528] and iron(III)[529,530] are also frequently used linkers in these compounds, but generally catecholate terminated peptides are required for ring closure **(41)**. The major advantage of these cyclic metallopeptides is that they can induce α-helicity in short peptide fragments and can be used as models of the active sites of metalloproteins as reported for thermolysine.[526] Some further examples of these metallomacrocycles have already been discussed in the previous paragraphs.[463–468]



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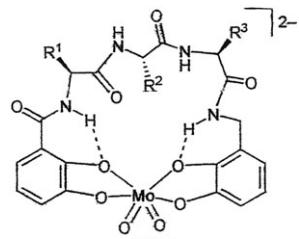
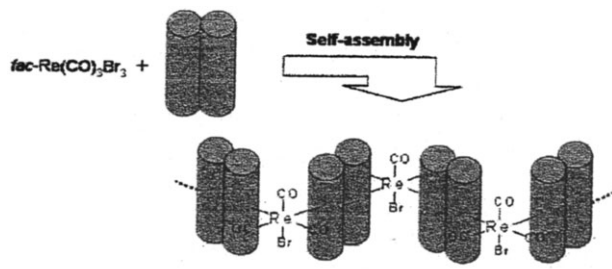


(41)

Molecular and/or chiral recognition of amino acids and peptides have become increasingly important in amino acid sequencing and in the study of protein function and solution structure. The corresponding studies cover the application of various macrocyclic ligands or other metal centered receptors linked to a rigid peptide backbone.[532–534] Peptide based electron transfer systems have also been designed in which the specific positions of the redox active metal complexes appended to either an α-helix or an α-helical coiled-coil.[535–537] In another study, metal-peptide nano-assemblies have been designed **(42)** in which the directional bonding properties of an octahedral rhenium complex are used to orient the self-assembling of peptide coiled-coils in a predetermined fashion.[538]



(42)

There is an increasing number of publications showing that various peptide complexes can be efficiently used as catalysts or reactants in different biochemical reactions. A 20-mer peptide, comprising an EF-hand calcium-binding sequence, was shown to bind europium(III) and promote ester cleavage in a pH-dependent manner.[539] Similar observations have been reported for several copper-metallated diglycine conjugates[540] and for zinc(II) complexes of dipeptides composed of two α,ω-diamino acid residues whose side chain amino groups were converted to dipicolylamino derivatives.[541] The superoxide dismutase activity of some copper(II) complexes of peptides of histidine has already been discussed in the previous paragraphs.[354,413,418,419] The interaction of metal complexes with DNA may help

to develop new anticancer drugs and other chemotherapeutic agents. Metal complexes of peptides and peptide conjugate ligands are also increasingly used in these studies.[542–544] Moreover, it has been reported that some peptide complexes can promote sequence selective DNA cleavage.[545,546]

The synthesis and characterization of rhenium and technetium complexes of peptides and related ligands as effective chelating and transporting agents of radionuclides have been the subject of continuous interest. Most of these publications can be found in more specific reviews focused on the biomedical applications and mentioned here are only those which describe the coordination behavior of the molecules in detail.[547–556] A ReO[N_3S]-type oxorhenium(V) complex of the decapeptide (RGDSCRGDSY) has been prepared and evaluated for possible application as a target-specific radiotherapeutic agent for the treatment of malignant melanoma. The amide nitrogen atoms of D(3) and S(4) and the amide and thiolate donors of C(5) residues were identified as the metal binding sites.[547] The binding of thiolate sulfur and preceeding amide nitrogen donor atoms was suggested in some other $[ReO]^{3+}$ and $[TcO]^{3+}$ complexes of peptides,[548–552] and there are publications on the role of histidyl residues[553] and nitrido complexes of technetium.[554]

A rapidly growing area in the applications of metal ion-peptide interactions is the development of new electrochemical, fluorescent or other optical sensors for analytical purposes.[557–561] The modification of an electrode with the tripeptide GGH has been described and used for the detection of copper with high selectivity and sensitivity.[557] The application of another peptide of histidine (β-alanylhistidine) was suggested for similar purposes in another study.[558] The pentapeptide, GHLLC, and CdS quantum dots were successfully combined to develop a highly sensitive and selective system for the detection of copper(II) and silver(I).[559] The development and physico-chemical properties of fluorescent sensors for the detection of copper(II)[560] and zinc(II)[561] with high sensitivity have also been reported.

The possibility of the industrial and/or environmental applications of metal ion peptide interactions or the complexes themselves has also been significantly increased in the period of coverage. A new patent has been registered for the application of gold-binding proteins or peptides for detection and/or recovery of gold from ores.[562] The high concentrations of metal ions in biological systems are often connected to the increased concentrations of reactive oxygen species (ROS). Metal-binding peptides have been suggested for the reduction of oxidative damage caused by these reactive oxygen species in animals.[563,564] The application of peptide complexes in the fabrication of noble metal nanoclusters[565] and nanotubes[566] is also promising and rapidly growing area of metallopeptide chemistry.

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