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Amino Acids Peptides and Proteins

VOLUME 16

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Amino Acids, Peptides, and Proteins

Volume 16

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Amino Acids, Peptides, and Proteins

Volume 16

A Review of the Literature Published during 1983

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Preface

This sixteenth Report reviews papers published during 1983 that are relevant to the chemistry of amino acids, peptides, and proteins. The content and format generally follow those of Volume 15. No chapter on Inorganic Aspects appears, because it has been customary to review this sector in alternate years only.

Coverage of Primary Structure proved impossible to arrange, as did the inclusion of separate subsections devoted to Nuclear Magnetic Resonance and Fluorescence. These gaps are obviously unsatisfactory features, which give us pain. Despite the gaps and an increasingly (excessively, even) compressed style we have still exceeded our allocation of pages, and it has become clear that we have been fighting a losing battle in our efforts to deal rapidly and exhaustively with a relentlessly expanding volume of literature. The present Report is the last that will attempt to review such a broad front. Its successor will concern itself more specifically with the chemistry of amino acids and peptides – a retrograde step, we admit, but inevitable. The field of review established in the early Reports in the series has grown beyond containment in a single volume without self-defeating selectivity and crowding.

One of the most important publications to appear during the year was the report of the I.U.P.A.C.-I.U.B. Joint Commission on Biochemical Nomenclature giving its 1983 recommendations for amino acid and peptide nomenclature and symbolism. Those who have thrashed out these recommendations with such patience and meticulous attention to detail have done the subject a great service. Whilst we make no claim to complete innocence from the occasional aberration ourselves, we urge everyone in the field to conform.

Balliol College, Oxford JOHN JONES
November 1984

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Abbreviations

Abbreviations for amino acids and their use in the formulations of derivatives follow, with rare exceptions, the 1983 Recommendations of the I.U.P.A.C.–I.U.B. Joint Commission on Biochemical Nomenclature, which are reprinted in full as an Appendix at the end of this volume.

Other abbreviations that have been used are listed here or are defined in the text and table footnotes.

Ac	acetyl
Acm	acetamidomethyl
Ad	adamantyl
Adoc	adamantyloxycarbonyl
Adpoc	1-(1-adamantyl)-1-methylethoxycarbonyl
AMP	adenosine 5′-monophosphate
Aoc	t-amyloxycarbonyl
Aox	4,5-dianisoyl-4-oxazolin-2-one amino acid derivative
Asu	α-aminosuberic acid
Asx	aspartic acid or asparagine (not yet determined)
atm	atmosphere
ATP	adenosine 5′-triphosphate
Azoc	2-(4-phenylazophenyl)isopropyloxycarbonyl
Beoc	2-bromoethyloxycarbonyl
Boc	t-butoxycarbonyl
Bom	benzyloxymethyl
Bpoc	2-(4-biphenylyl)isopropoxycarbonyl
Br·Bom	4-Br-benzyloxymethyl
BSA	bovine serum albumin
BTCO	1-benzotriazolylcarbonyl
Btm	benzylthiomethyl
Bu^t	t-butyl
Bzh	benzhydryl (diphenylmethyl)
$Bzh(OMe)_2$	4,4′-dimethoxybenzhydryl
Bzl	benzyl
Bzl(4-Cl)	4-chlorobenzyl
$Bzl(2,6\text{-}Cl_2)$	2,6-dichlorobenzyl
Bzl(4-CN)	4-cyanobenzyl
$Bzl(NO_2)$	4-nitrobenzyl
$Bzl(2\text{-}NO_2)$	2-nitrobenzyl
Bzl(OMe)	4-methoxybenzyl
Cac	9-carbazolylcarbonyl
c.d.	circular dichroism
Cha	cyclohexylamine

c.i.-m.s.	chemical-ionization mass spectrometry
CLIP	adrenocorticotropic hormone (18–39)
Cm	carboxymethyl
Cmc	*S*-carboxymethylcysteine
CoA	coenzyme A
Cox	4,5-di-(4-chlorophenyl-4-oxazoline-2-one) derivative
Cpe	cyclopentyl
CPh_2Py	diphenyl-4-pyridylmethyl
DCCI	dicyclohexylcarbodi-imide
Dcha	dicyclohexylamine
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
Ddz	3,5-dimethoxy-(αα-dimethyl)benzyloxycarbonyl
Dha·NHEt	dehydroalanylethylamide
DHCH	1,2-dihydroxycyclohex-1,2-ylene
DMA	dimethylacetamide
Dmb	2,4-dimethoxybenzene sulphonyl
DMCBzl	dimethylcarbamoylbenzyl
DMCZ	dimethylcarbamoylbenzyloxycarbonyl
DMF	*N*,*N*-dimethylformamide
Dmoc	1,3-dithian-2-yl-methoxycarbonyl
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
Dnp	2,4-dinitrophenyl
2,4-Dnps	2,4-dinitrosulphenyl
Dnpy	3,5-dinitro-1-(*p*-nitrophenyl)-4-pyridone
Dns	1-dimethylaminonaphthalene-5-sulphonyl (dansyl)
Dopa	3,4-dihydroxyphenylalanine
DP	degree of polymerization
DPA	diphenylacetyl
Dpp	diphenylphosphinoyl
Dppm	diphenyl-4-pyridylmethyl
DPtd	4,6-diphenylthieno[3,4-*d*][1,3]dioxal-2-one 5,5-dioxide
DTNB	5,5′-dithiobis-(2-nitrobenzoic acid)
Dts	dithiosuccinoyl
Ec	ethylcarbamoyl
edta	ethylenediaminetetra-acetate
EEDQ	2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline
e.i.-m.s.	electron-impact ionization mass spectrometry
En	ethylenediamine
e.n.d.o.r.	electron nuclear double resonance
e.p.r.	electron paramagnetic resonance
e.s.r.	electron spin resonance
Et	ethyl
f.a.b.	fast-atom bombardment
Fal	hexafluorovaline
f.d.	field desorption
Fm	9-fluorenylmethyl

Fmoc	9-fluorenylmethoxycarbonyl
GABA	γ-aminobutyric acid
Gal	galactose
g.c.-m.s.	gas chromatograph–mass spectrometer combination
g.l.c.	gas–liquid chromatography
Glc	glucose
Glp	2-pyrrolidone-5-carboxylic acid
Glx	glutamic acid or glutamine (not yet determined)
GS	gramicidin S
GTP	guanosine 5′-triphosphate
HMPA	hexamethylphosphoric triamide
HOBt	hydroxybenzotriazole
h.p.l.c.	high-performance liquid chromatography
Iboc	isobornyloxycarbonyl
i.r.	infrared
LDA	lithium di-isopropylamide
Mal	maleoyl
Man	mannose
Mbh	4,4-dimethoxybenzhydryl
Mbs	4-methoxybenzenesulphonyl
Mds	4-methoxy-2,6-dimethylbenzenesulphonyl
Me	methyl
Mea	mercaptoethylamine
$MePh_2Peoc$	2-methyldiphenylphosphinioethyloxycarbonyl
Mhoc	1-methylcyclohexylcarbonyl
Moc	methoxycarbonyl
Mpt	dimethylphosphothioyl
m.s.	mass spectrometry
Msc	2-(methylsulphonyl)ethoxycarbonyl
Mtb	2,4,6-trimethoxybenzene sulphonyl (mesitylene sulphonyl)
Mtc	2-methylthioethoxycarbonyl
MTM	methylthiomethyl
Mtr	2,3,6-trimethyl-4-methoxybenzenesulphonyl
NAD	nicotinamide-adenine dinucleotide
NCA	*N*-carboxyanhydride
Nma	maleimido
Nmps	4-methyl-2-nitrosulphenyl
n.m.r.	nuclear magnetic resonance
n.O.e.	nuclear Overhauser effect
Np	4-nitrophenyl
NPh	phthalimido
Nps	2-nitrophenylsulphenyl
Npys	3-nitropyridine-2-sulphenyl
Nsu	succinimido
OHFP	hexafluoroisopropyl ester
ONp	4-nitrophenyl ester
ONp(*o*)	2-nitrophenyl ester

ONp·Pic	2-nitro-4-(4-picolyloxycarbonyl)phenyl ester
ONSu	succinimido ester
OPcp	pentachlorophenyl ester
OPfp	pentafluorophenyl ester
OPic	4-picolyl ester
Opop	2-(phenacyloxy)phenyl ester
o.r.d.	optical rotatory dispersion
OTAT	thiazoline-2-thione ester
OTce	2,2,2-trichloroethyl ester
OTcp	2,4,5-trichlorophenyl ester
PAAC	isopropylidene aminoxycarbonyl
Pac	phenacyl
PAC	phenylacetyl
Pam	4-(hydroxymethyl)phenyl acetamidomethyl (resin)
PChd	3,5-di-t-butyl-4-oxo-1-phenyl-2,5-cyclohexadienyl
PEA	phenylethylamine
Peoc	2-triphenylphosphinioethyloxycarbonyl
Ph(SMe)	*p*-methylthiophenyl
Pic	4-picolyl
Picoc	4-picolyloxycarbonyl
Pipoc	piperidino-oxycarbonyl
Piv	pivaloyl
Pme	pentamethylbenzenesulphonyl
Pms	*p*-tolylmethylsulphonyl
Poc	cyclopentyloxycarbonyl
Poly(A)	poly(adenylic acid)
Ppoc	phenylisopropoxycarbonyl
Ppt	diphenylphosphinothioyl
Pth-Gly	the phenylthiohydantoin derived from glycine, *etc.*
py	pyridine
Pz	*p*-phenylazobenzyloxycarbonyl
SBu^t	t-butylthio
SCB	t-butyloxycarbonylsulphenyl
Scm	carboxymethylsulphenyl
SDS	sodium dodecyl sulphate
SPr^i	isopropylthio
Sub	5-dibenzosuberyl
Tac	toluene-*p*-sulphonylaminocarbonyl
tBa	t-butylammonium
TCBoc	2,2,2-trichloro-1,1-dimethylethoxycarbonyl
Tcp	2,4,5-trichlorophenyl
Tcroc	2-CF_3-chromonylmethylenecarbonyl
Tcrom	2-CF_3-chromonylmethyl
Tfa	trifluoroacetyl
Thp	tetrahydropyranyl
Tht	thiazolidine-2-thione
t.l.c.	thin-layer chromatography

Tmb	2,4,6-trimethylbenzyl
Tmeda	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine
Tmg	tetramethylguanidine
TMSE	2-trimethylsilylethoxycarbonyl
Tnps	2,4,6-trinitrophenylsulphenyl
Tos	toluene-*p*-sulphonyl
TRH	thyrotropin-releasing hormone
Troc	2,2,2-trichloroethoxycarbonyl
Trt	triphenylmethyl
Tse	2-(toluene-*p*-sulphonyl)ethyl
u.v.	ultraviolet
Xan	9-xanthydryl
Z	benzyloxycarbonyl
Z(2-Br)	2-bromobenzyloxycarbonyl
Z(OMe)	4-methoxybenzyloxycarbonyl
Ztf	1-benzyloxycarbonylamino-2,2,2-trifluoroethyl

1
Amino Acids

BY G. C. BARRETT

1 Introduction

The literature searched to generate this chapter continues to be centred on the chemical journals and monographs, though taking in the biochemical and biological literature where this deals with analytical advances and material relevant to chemical studies.

Textbooks and Reviews. – A comprehensive coverage is offered by a multi-author text[1] that concentrates on the advances in knowledge of the chemistry and biochemistry of the amino acid in the period following coverage given in Greenstein's and Winitz's classic treatise.[2] Several chapters of 'Methods in Enzymology'[3] will be of general interest to readers of this chapter, as will reviews of new natural amino acids,[4] uses and biochemistry of amino acids,[5,6] and methods for their synthesis.[6] More specialized reviews concern valine,[7] tryptophan,[8] biochemistry of sulphur-containing amino acids,[9] ketimine derivatives of sulphur-containing amino acids,[10] electrophoresis of amino acids,[11] motion in solid amino acids,[12] and nitrogen interconversion and amino acid metabolism in germinating seedlings.[13]

2 Naturally Occurring Amino Acids

Occurrence of Known Amino Acids. – A narrower outlook than in previous volumes can be detected in the following section, which lacks mention of amino

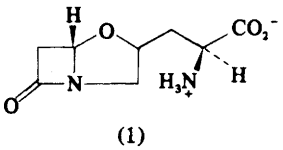
[1] 'Chemistry and Biochemistry of the Amino Acids', ed. G. C. Barrett, Chapman and Hall, London, 1984.
[2] J. P. Greenstein and M. Winitz, 'Chemistry of the Amino Acids', Wiley, New York, 1961.
[3] 'Methods in Enzymology', Vol. 91, Part I (Enzyme Structure), ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, 1983: 'γ-Carboxyglutamic Acid', P. A. Price, p. 13; '*N*-Methylamino Acids', M. Elzinga and N. Alonzo, p. 8; 'Buffer Systems for the Amino Acid Analyzer', C. H. W. Hirs, p. 3 (see also refs. 426, 435, and 436).
[4] I. Wagner and H. Nusso, *Angew. Chem.*, 1983, **95**, 827.
[5] B. Hoppe and J. Martens, *Chem. Unserer Zeit*, 1983, **17**, 41.
[6] V. S. Shpak and I. Ya. Tyuryaev, *Vestn. Akad. Nauk S.S.S.R.*, 1983, 107.
[7] A. S. Polyanskaya, I. Kalnins, N. I. Aboslalova, and G. I. Prigorodov in 'Sintesy Issled. Nitrosoedin. Aminokislot', ed. G. V. Nekrasova, Leningr. Gos. Pedagog. Inst., Leningrad, 1983, p. 51.
[8] B. Witkop, *Heterocycles*, 1983, **20**, 2059.
[9] A. J. L. Cooper, *Ann. Rev. Biochem.*, 1983, **52**, 263.
[10] D. Cavallini, *Progr. Clin. Biol. Res.*, 1983, **125**, 355.
[11] Z. Deyl in 'Electrophoresis: Part B', ed. Z. Deyl, Elsevier, Amsterdam, 1983, p. 55.
[12] M. A. Keniry, R. L. Smith, H. S. Gulowsky, and E. Oldfield in 'Structure and Dynamics: Nucleic Acids and Proteins', ed E. Clementi and R. H. Sarma, Adenine Press, Guilderland, New York, 1983, p. 435.
[13] P. J. Lea and K. W. Joy, *Recent Adv. Phytochem.*, 1983, **17**, 77.

acids as constituents of meteorites or Earthly geological sources but otherwise covers non-routine work with uncommon amino acids in plant and animal sources. *cis*-4-Hydroxy- and 2,4-*cis*-4,5-*trans*-dihydroxy-pipecolic acids are present in leaves of *Calliandra pittieri*,[14] and isoasparagine makes its first natural appearance in *Chara corallina*.[15] 2,6-Diamino-7-hydroxyazelaic acid, a constituent of the edeine antibiotics, has the (2*R*,6*S*,7*R*) configuration.[16] N^{ϵ}-Methyl-lysine is a constituent of flagellins from *Proteus morganii*,[17] and *N*-trimethylalanine appears at the N-terminus of myosin light chains.[18] The structure of the cross-linking residue of collagen, pyridinoline (see Vol. 14, p. 3), has been confirmed.[19]

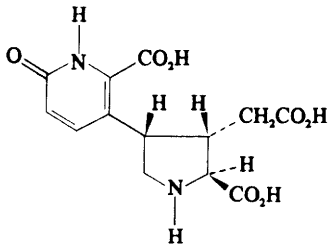
A crop of papers describes *N*-substituted amino acids of particular interest either in themselves or in their location: L-γ-glutamyl-*cis*-3-aminoproline in *Morchella esculenta*,[20] 4′-*O*-diacylglyceryl-*N*-trimethylhomoserine from fronds of the fern *Adiantum capillus veneris* (the first report of this amino acid in a vascular plant, previously detected in algae),[21] and a role of *N*-acetyl-L-aspartic acid as one heat-stable factor essential for the conversion of lignoceric acid to cerebronic acid and glutamic acid, catalysed by a rat-brain particulate preparation.[22]

New Natural Amino Acids. – *Streptomyces clavuligerus* produces the new clavam $R_0$22-5417 (1), as well as other β-lactams.[23] New kainoids [acromelic acids A and B, *i.e.* (2) and the corresponding 5′-pyridin-2′-one-6′-carboxylic acid, respectively] have been isolated from *Clitocybe acromelalga*.[24]

Mutant strains of *Neurospora crassa* that accumulate prephenic and arogenic acids (see Vol. 13, p. 3) also produce spiro-L-arogenate, the previously unknown lactam of arogenic acid.[25]



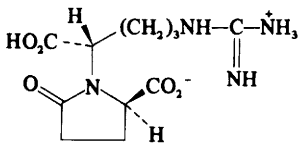
(1)



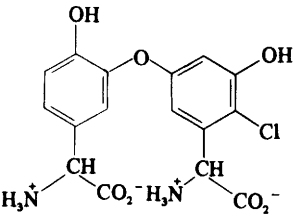
(2)

[14] J. T. Romeo, L. A. Swain, and A. B. Bleecker, *Phytochemistry*, 1983, **22**, 1615.
[15] K. Sakano, T. Shimmen, S. Hatanaka, and M. Tazawa, *Phytochemistry*, 1983, **22**, 2313.
[16] J. Gumieniak, H. Wojciechowska, and E. Borowski, *Pol. J. Chem.*, 1981, **55**, 1519.
[17] B. S. Baker, S. E. Smith, and M. W. McDonough, *Microbios Lett.*, 1983, **23**, 7.
[18] G. D. Henry, D. C. Dalgarno, B. A. Levine, and I. P. Trayer, *Biochem. Soc. Trans.*, 1982, **10**, 362.
[19] S. P. Robins and A. Duncan, *Biochem. J.*, 1983, **215**, 175; S. P. Robins, *ibid.*, p. 167.
[20] M. Moriguchi, K. Kimura, and S. Hatanaka, *Nippon Kingakkai Kaiho*, 1983, **24**, 191.
[21] N. Sato and M. Furuya, *Plant Cell Physiol.*, 1983, **24**, 1113.
[22] H. Shigematsu, N. Okamura, H. Shimeno, Y. Kishimoto, L. S. Kan, and C. Fenselau, *J. Neurochem.*, 1983, **40**, 814.
[23] D. L. Pruess and M. Kellett, *J. Antibiot.*, 1983, **36**, 208; R. H. Evans, H. Ax, A. Jacoby, T. H. Williams, E. Jenkins, and J. P. Scannell, *ibid.*, p. 213.
[24] K. Konno, H. Shirahama, and T. Matsumoto, *Tetrahedron Lett.*, 1983, **24**, 939.
[25] L. O. Zamir, E. Jung, and R. A. Jensen, *J. Biol. Chem.*, 1983, **258**, 6492.

Metabolites from crown-gall tumours induced by *Agrobacterium tumefaciens* continue to provide new amino acids, helping to fuel the interest in this topic, which is clearly of broader fundamental importance. Strains that utilize neither octopine nor norpaline produce tumours that contain the lactam of N^2-(1,3-dicarboxypropyl)-L-leucine, for which the name leucinopine is suggested.[26] Similar studies have revealed the presence of pyronopaline (3) in crown-gall tumour tissue.[27]



(3)



(4)

New Amino Acids from Hydrolysates. – This section encompasses the literature that describes new amino acids found as constituents of peptides, peptidoglycans, and related natural products. A new type of carbohydrate linkage is seen in N^{β}-asparaginylglucose, a constituent of *Halobacterium* cell-wall glycoprotein[28] (a related amino acid is found in hen ovalbumin[29]). *threo*-β-Hydroxyornithine (probably the D-enantiomer[30]) is a constituent of a peptidoglycan from *Corynebacterium Co112*.[30] *erythro*-β-Hydroxyaspartic acid is present in a light chain of bovine protein C, an anticoagulant Vitamin K-dependent plasma protein[31] (eleven γ-carboxyglutamic acid residues are also present[32]).

Isolation and identification of hypusine [N'-(4-amino-2-hydroxybutyl)lysine] and its 2-deoxy analogue from brain tissue have been fully documented.[33] In another continuing study of novel amino acids as constituents of larger structures, actinoidinic acid and a related arylglycine (4) are formed by hydrolysis of the aglycone component of the glycopeptide antibiotic A35512B, the absolute configuration of (4) being (*R*) for the *p*-hydroxyphenylglycine moiety and (*S*) for the *m*-hydroxylated moiety.[34] Rhizonin A, from *Rhizopus microsporus*, contains *N*-methyl-3-(3-furyl)-L-alanine.[35]

[26] C. C. Chang, C. M. Chen, B. R. Adams, and B. M. Trost, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 3573.
[27] L. M. Hall, J. L. Schrimsher, and K. B. Taylor, *J. Biol. Chem.*, 1983, **258**, 7276.
[28] F. Wieland, R. Heitzer, and W. Schaefer, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 5470.
[29] H. Nomoto and Y. Inoue, *Eur. J. Biochem.*, 1983, **135**, 243.
[30] K. H. Schleifer, I. Hayn, H. P. Seidl, and J. Firl, *Arch. Microbiol.*, 1983, **134**, 243.
[31] T. Drakenberg, P. Fernlund, P. Roepstorff, and J. Stenflo, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 1802.
[32] B. A. McMullen, K. Fujikawa, W. Kisiel, T. Sasagawa, W. N. Howald, E. Y. Kwa, and B. Weinstein, *Biochemistry*, 1983, **22**, 2875.
[33] M. H. Park, H. L. Cooper, and J. E. Folk, *Methods Enzymol.*, 1983, **94**, 458.
[34] C. M. Harris and T. M. Harris, *Tetrahedron*, 1983, **39**, 1661.
[35] P. S. Steyn, A. A. Tuinman, F. R. van Heerden, P. H. van Rooyen, P. L. Wessels, and C. J. Rabie, *J. Chem. Soc., Chem. Commun.*, 1983, 47.

Crosslinking amino acid residues in proteins having important physiological functions continue to stimulate interest. Both known[19] and novel[36] crosslinking residues feature in the 1983 literature, the latter represented in the presence of N^{π}-histidino-alanine (as well as the previously known N^{τ}-isomer) in calcium-binding phosphoprotein particles from the clam *Rangia cuneata*.[36]

3 Synthesis of Amino Acids

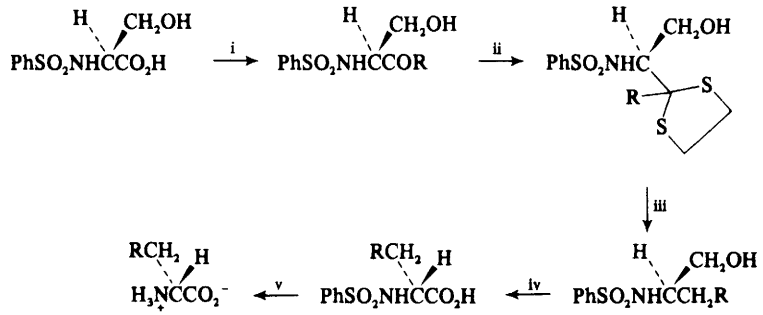
General Methods. – Standard methods employing ammonia in reactions with keto acids to give *N*-acylamino acids as well as amino acids[37] (see also Vol. 15, p. 4) and with 1,1-dichloro-oxiranes to give amino acids[38] and a related use of Me_3N in a synthesis of DL-carnitine by aminolysis of $ClCH_2CH(OH)CH_2CO_2Et$ followed by hydrolysis[39] have been reported. Other standard synthetic procedures used are alkylation of diethyl acetamidomalonate[40,109,130,169] (to give β-pyrazinyl-L-alanine[40]) and of diethyl formamidomalonate,[41] also Strecker[42] and hydantoin[43] alkylation. Alkylation of glycine derivatives is being taken up in more laboratories for the synthesis of α-amino acids.

A later section ('Reactions of Amino Acids') of this chapter also happens to include some general methods of synthesis of amino acids, possibly not the primary objective of the authors concerned, but a growing number of papers is directed at the use of readily available amino acids as starting materials for the synthesis of other amino acids. L-Serine is the starting material in a chirally efficient synthesis of D-α-amino acids $RCH_2CH(\overset{+}{N}H_3)CO_2^-$. *N*-Benzenesulphonyl-L-serine reacts with organo-lithium and -magnesium compounds at −78 °C to give optically pure amino ketones, from which the D-amino acid is obtained through conventional stages (Scheme 1).[44] Alkylation of a ω-iodo or -bromo acid with four equivalents of an organocuprate R_2CuLi gives excellent yields of the substitution products of high optical purity.[45]

A stereoselective synthesis of γ-hydroxyamino acids from α-nitrosoalk-2-enoic amides $MeCH{=}C(NO)CONHBu^t$ involves condensation with an aldehyde.[46]

A new general β-amino acid synthesis is based on the alkylation of *N*-benzyloxyimines with ketene silylacetals [$R^1CH{=}NOCH_2Ph + R^2R^3C{=}C(OR^4)OSiMe_3 \rightarrow PhCH_2ONHCHR^1CR^2R^3CO_2R^4$].[47]

[36] R. L. Sass and M. E. Marsh, *Biochem. Biophys. Res. Commun.*, 1983, **114**, 304.
[37] H. Yamagawa, Y. Makino, K. Sato, M. Nishizawa, and F. Egami, *Adv. Space Res.*, 1983, **3**, 69.
[38] L. Yu and M. Yan, *Gaodeng Xuexiao Huaxue Xuebao*, 1983, **4**, 213 (*Chem. Abstr.*, 1984, **100**, 7086).
[39] Y. Chen, J. Chen, and K. Qian, *Hunan Yixueyuan Xuebao*, 1983, **8**, 82 (*Chem. Abstr.*, 1983, **99**, 122 847).
[40] C. Petermann and J. L. Fauchere, *Helv. Chim. Acta*, 1983, **66**, 1513.
[41] L. Yu and H. Liang, *Beijing Shifan Daxua Xuebao, Ziran Kexueban*, 1983, 94 (*Chem. Abstr.*, 1983, **99**, 105 665).
[42] S. Yu. Sizov, L. V. Semenova, and N. P. Utrobin in ref. 7, p. 38, 42.
[43] D. Mostowicz, W. Abramski, and C. Belzecki, *Pol. J. Chem.*, 1981, **55**, 1387.
[44] P. J. Maurer, H. Takahata, and H. Rapoport, *J. Am. Chem. Soc.*, 1984, **106**, 1095.
[45] A. Bernardini, A. El Hallaoui, R. Jacquier, C. Pigiere, P. Viallefont, and J. Bajgrowicz, *Tetrahedron Lett.*, 1983, **24**, 3717.
[46] B. J. Banks, A. G. M. Barrett, M. A. Russell, and D. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1983, 873.
[47] K. Ikeda, K. Achiwa, and M. Sekiya, *Tetrahedron Lett.*, 1983, **24**, 4707.



Reagents: i, RLi and RMgBr; ii, $HSCH_2CH_2SH$ and $BF_3 \cdot Et_2O$; iii, Raney Ni; iv, O_2–PtO_2; v, 48% aq. HBr

Scheme 1

Asymmetric Synthesis of Amino Acids. – Several reviews have appeared,[48–50] including coverage of non-enzymic transamination[49] and catalysed asymmetric hydrogenation.[50]

Much of the recent literature on this topic reports extensions of studies of groups active over several years. Reductive aminolysis of 4-alkylidene- and -arylidene-oxazolin-5-ones,[51] aminolysis of 4-benzyl- or isopropyl-oxazolin-5-ones,[52] and catalysed asymmetric hydrogenation of *Z*- and *E*-isomers of *N*-benzoyl-α-amino-β-methyl-β-ethylacrylate[53] have raised interest mainly of a mechanistic nature, since enantiomeric discrimination in these reactions is usually low owing to accompanying racemization. The other work based on catalysed asymmetric hydrogenation,[54–57] though from different laboratories, employs rhodium(I)–chiral phosphine catalysis and includes pessimistic assessment[57] of the Ruch and Ugi proposals that enantioselectivity of this process can be predicted. The alternative approach, in which hydrogenation catalysed by achiral means

[48] U. Schöllkopf, *Top. Curr. Chem.*, 1983, **109**, 65.

[49] H. Kuzuhara, *Yuki Gosei Kagaku Kyokaishi*, 1983, **41**, 134.

[50] W. S. Knowles, *Acc. Chem. Res.*, 1983, **16**, 106; B. D. Vineyard, W. S. Knowles, and M. J. Sabacky, *J. Mol. Catal.*, 1983, **19**, 159.

[51] G. V. Chel'tsova, E. I. Karpeiskaya, L. N. Kaigorodova, and E. I. Klabunovskii, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1983, 65; I. E. Khatskevich, I. K. Kalnin, E. I. Karpeiskaya, and E. I. Klabunovskii, *ibid.*, p. 359, 366; E. S. Levitina, L. F. Godunova, E. I. Karpeiskaya, and E. I. Klabunovskii, *ibid.*, p. 1740.

[52] G. V. Chel'tsova, E. I. Karpeiskaya, E. I. Klabunovskii, and E. D. Lubuzh, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1983, 59; L. F. Godunova, E. S. Levitina, E. I. Karpeiskaya, E. I. Klabunovskii, and E. D. Lubuzh, *ibid.*, p. 1733.

[53] N. D. Zubareva, I. E. Khatsevich, T. I. Kuznetsova, Sh. G. Bitiev, I. Kalnins, E. I. Karpeiskaya, A. A. Vedenyapin, and E. I. Klabunovskii, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1983, 477.

[54] D. Sinou, D. Lafont, G. Descotes, and T. Dayrit, *Nouv. J. Chim.*, 1983, **7**, 291.

[55] C. Cativiela, J. A. Mayoral, E. Melendez, R. Uson, L. A. Oro, and M. T. Pinillos, *React. Kinet. Catal. Lett.*, 1982, **21**, 173.

[56] M. Yatagai, M. Zama, T. Yamagishi, and M. Hida, *Chem. Lett.*, 1983, 1203.

[57] H. Brunner, B. Schönhammer, B. Schönhammer, and C. Steinberger, *Chem. Ber.*, 1983, **116**, 3529.

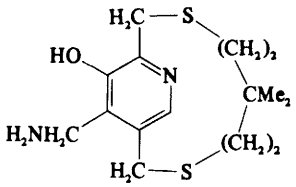
(*e.g.* Pd[58]) is applied to a chiral 3-alkylidene dioxopiperazine, leads to high chiral induction (90.1–98.4%) in most cases.[58] Stereoselective bromination of the same starting material using *N*-bromosuccinimide in methanol[59] gives 3-bromoalkyl-3-methoxypiperazinediones in less substantial enantiomeric excesses.

Several more papers from the Schöllkopf group[60–68] extend the use of bis-lactim ethers derived from dioxopiperazines into synthesis of (*R*)-(−)-isovaline,[65] (*R*)-α-methyl-*S*-benzylcysteine,[66] (*R*)-serine derivatives,[67] and (*R*)-β-fluorovaline and related 2-amino-3-fluoroalkanoic acids.[68] The principle employed in this approach is also exemplified in the asymmetric alkylation of the carbanion derived from a chiral 2-methoxy-3-substituted Δ^1-1,4-tetrahydro-oxazin-3-one.[69]

The remaining papers considered here extend established principles either in the control exerted by a nearby chiral centre over alkylation adjacent to nitrogen[70–73] or in enantioselective decarboxylation of an α-alkyl-α-amino-malonate.[74] In the latter area,[74] employing chiral cobalt(III) complexes (see Vol. 14, p. 8), and in the alkylation of amidines formed by the reaction of (*S*)-*N*-dimethoxymethylprolinol methyl ether with a DL-α-amino acid[73] useful levels of enantiomeric purity are achieved only in the second of these two studies. Schiff bases formed either from chiral carbonyl compounds (D-galacto-dialdehyde[70] or 2-hydroxypinan-3-one[71]) by reaction with a DL-amino acid ester or from a chiral pyridoxamine analogue and a keto acid[72] continue to be used in the asymmetric synthesis of amino acids; alkylation of the DL-amino acid derivatives gives satisfactory enantiomeric purities leading to α-alkyl analogues of the common L-amino acids,[70, 71] while enantiomeric excesses between 60 and 96% have been reported[72] for hydrolysis of the Schiff base formed between an enantiomer of compound (5) and a keto acid in the presence of Zn^{2+} ions.

Models for Prebiotic Synthesis of Amino Acids. – Several reviews of the classic Miller and Urey system for synthesis of amino acids from simple molecules (for example combinations of H_2, CH_4, H_2O, N_2, and NH_3 subjected to a spark

[58] Y. Hashimoto, H. Aoyagi, M. Waki, T. Kato, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1983, **21**, 11.
[59] C. G. Shin, Y. Yonezawa, Y. Sato, T. Nakano, and J. Yoshimura, *Heterocycles*, 1983, **20**, 405.
[60] U. Schöllkopf and H. J. Neubauer, *Synthesis*, 1982, 861.
[61] U. Schöllkopf and Y. C. Chiang, *Synthesis*, 1982, 864.
[62] J. Nozulak and U. Schöllkopf, *Synthesis*, 1982, 866.
[63] U. Schöllkopf, J. Nozulak, and U. Groth, *Synthesis*, 1982, 868.
[64] U. Schöllkopf, *Tetrahedron*, 1983, **39**, 2085.
[65] U. Schöllkopf and R. Lonsky, *Synthesis*, 1983, 675.
[66] U. Groth and U. Schöllkopf, *Synthesis*, 1983, 37.
[67] U. Schöllkopf, U. Groth, M. R. Gull, and J. Nozulak, *Liebigs Ann. Chem.*, 1983, 1133.
[68] U. Groth and U. Schöllkopf, *Synthesis*, 1983, 673.
[69] W. Hartwig and U. Schöllkopf, *Liebigs Ann. Chem.*, 1982, 1956.
[70] I. Hoppe, U. Schöllkopf, and R. Tolle, *Synthesis*, 1983, 789.
[71] J. A. Bajgrowicz, B. Cossec, C. Pigiere, R. Jacquier, and P. Viallefont, *Tetrahedron Lett.*, 1983, **24**, 3721.
[72] Y. Tachibana, M. Ando, and H. Kuzuhara, *Chem. Lett.*, 1982, 1765, 1769.
[73] M. Kolb and J. Barth, *Liebigs Ann. Chem.*, 1983, 1668.
[74] M. J. Jun, N. M. Yoon, and C. F. Liu, *J. Chem. Soc., Dalton Trans.*, 1983, 999; *J. Coord. Chem.*, 1983, **12**, 279.



(5)

discharge) have appeared.[75] The new results seen in the recent literature continue to demonstrate the suitability of an increasing range of simple compounds in this process, experiments supporting the possible roles of newly identified members of the galactic chemistry set. U.v. irradiation of hydrazine, formaldehyde, and water over $CaCO_3$ in an anoxic environment generates amino acids and other compounds of biological interest,[76] while similar treatment of aqueous ammonium acetate creates glycine, alanine, β-alanine, and γ-aminobutyric acid (and possibly also serine, aspartic acid, and valine).[77] The infinite variety of adjustments that can be applied to the Miller–Urey system is not yet quite exhausted; a mixture of steam, N_2, CO_2, and NH_3 (1:1:1:0.1) streamed at elevated temperatures over a fluidized volcanic ash bed subjected to electric discharge gives serine, glycine, alanine, and glutamic acid as major amino acid products.[78]

Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids. – As in previous volumes, only representative citations can be offered to indicate the burgeoning literature covering fermentative production of protein amino acids. Section 16 (Fermentation and Bio-industrial Chemistry) of *Chem. Abstr.* offers easy access to most of the papers and patents in this field. The literature of the biosynthesis of amino acids also can only be covered in a superficial manner here. Many of the papers cited in this section concern the use of one L-amino acid as a substrate for microbial or enzymatic conversion into another. Production of L-lysine has been described,[79] as has the conversion of L-serine into L-tryptophan,[80] phenylalanine into L-Dopa,[81] L-glutamic acid into γ-aminobutyric acid,[82] and L-homocysteine into *S*-adenosyl-L-homocysteine employing beef-liver *S*-adenosyl-L-homocysteine hydrolase.[83]

[75] S. L. Miller and G. Schlesinger, *Adv. Space Res.*, 1983, **3**, 47; G. Schlesinger and S. L. Miller, *J. Mol. Evol.*, 1983, **19**, 376; A. Lazcano, J. Oro, and S. L. Miller, *Precambrian Res.*, 1983, **20**, 259.

[76] C. Folsome, A. Brittain, and M. Zelko, *Origins Life*, 1983, **13**, 49.

[77] M. Akaboshi, K. Kawai, H. Maki, K. Kawamoto, and Y. Honda, *Origins Life*, 1982, **12**, 339.

[78] G. A. Lavrent'ev, T. F. Strigunkova, Z. Yu. Rakilin, L. A. Piskunova, and I. A. Egorov, *Dokl. Akad. Nauk S.S.S.R.*, 1982, **267**, 756.

[79] A. Rutkov, *Acta Microbiol. Bulg.*, 1983, **13**, 33, 40.

[80] L. Nyeste, M. Pecs, B. Sevella, and J. Hollo, *Adv. Biochem. Eng., Biotechnol.*, 1983, **26**, 175; W. G. Bang, S. Lang, H. Sahm, and F. Wagner, *Biotechnol. Bioeng.*, 1983, **25**, 999.

[81] H. J. Wichers, T. M. Malingre, and H. J. Huizing, *Planta*, 1983, **158**, 482.

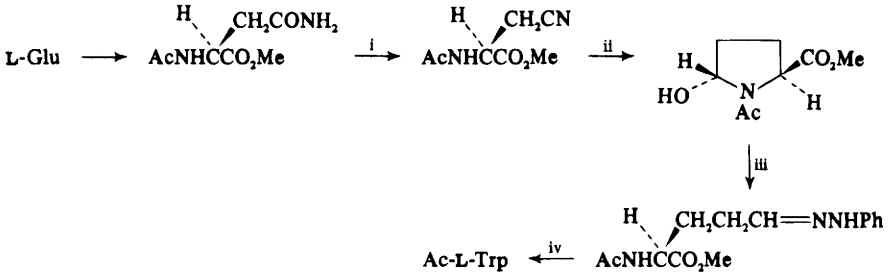
[82] R. Januseviciute, A. Pauliukonis, and D. Kazlauskas, *Khim. Prir. Soedin.*, 1983, 246.

[83] B. Chabannes, A. Gharib, L. Cronenberger, and H. Pacheco, *Prep. Biochem.*, 1983, **12**, 395.

Biosynthesis of phenylalanine and tyrosine from prephenic acid in *Streptomycetes*[84] and of β-(6-benzylaminopurin-9-yl)alanine from *O*-acetyl-L-serine and 6-benzylaminopurine in higher plants[85] and the unexpected finding that furanomycin arises from two acetate and one propionate unit[86] are typical of the range of biosynthetic work appearing in 1983.

A practical benefit arising from considerable differences in rates of aminoacylase-catalysed reactions is the separation of L-leucine from L-isoleucine by conversion into their *N*-acetyl derivatives and aminoacylase–cobalt(II)-catalysed hydrolysis; conditions have been found that allow L-leucine to crystallize out as the process continues.[87]

Laboratory syntheses of less common L-amino acids from protein amino acids are featured this year, as in most previous volumes in this series; conversion of one protein amino acid into another (synthesis of L-tryptophan from L-glutamic acid[88]) is a relatively rare laboratory operation. L-Glutamic γ-semi-aldehyde, formed from glutamic acid *via* *N*-acetyl-L-asparagine methyl ester (Scheme 2), gives a phenylhydrazone from which *N*-acetyl-L-tryptophan is obtained with negligible racemization.[88] L-Lysine serves as a source of L-pipecolic acid (N^{ϵ}-benzylidene derivative underwent N^{α}-benzylation; treatment with NaOBr then HBr debenzylation gave 19% L-pipecolic acid through treatment with Et_3N),[89] also as a source of L-α-aminoadipic acid (lysine, protected except for the side-chain amino group, underwent successive conversions $-CH_2NH_2 \rightarrow -CH_2NHN{=}NPh$ with $PhN_2^+BF_4^- \rightarrow CH_2OH \rightarrow CO_2H$ in 50% overall yield[90]). An alternative synthesis of DL-α-aminoadipic acid[91] in which hippuric acid is alkylated with



Reagents: i, tosyl chloride, pyridine; ii, H_2/Raney Ni; iii, $PhNHNH_2$; iv, refluxing 0.1M HCl; v, H_3O^+

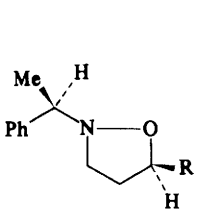
Scheme 2

[84] B. Keller, E. Keller, H. Goerisch, and F. Lingens, *Hoppe-Seyler's Z. Phyiol. Chem.*, 1983, **364**, 455.
[85] I. Murakoshi, C. Koide, F. Ikegami, and K. Nasu, *Chem. Pharm. Bull.*, 1983, **31**, 1777.
[86] R. J. Parry and H. P. Buu, *J. Am. Chem. Soc.*, 1983, **105**, 7446.
[87] J. Martens and H. Weigel, *Liebigs Ann. Chem.*, 1983, 2052.
[88] F. Masumi, H. Takeuchi, S. Kondo, K. Suzuki, and S. Yamada, *Chem. Pharm. Bull.*, 1982, **30**, 3831.
[89] H. Mihara, H. Aoyagi, M. Waki, T. Kato, and N. Izumiya, *Mem. Fac. Sci., Kyushu Univ., Ser. C*, 1983, **14**, 123.
[90] J. E. Baldwin, P. Harrison, and J. A. Murphy, *J. Chem. Soc., Chem. Commun.*, 1982, 818.
[91] N. H. Khan, M. Ali, and H. Panda, *J. Indian Chem. Soc.*, 1982, **59**, 1077.

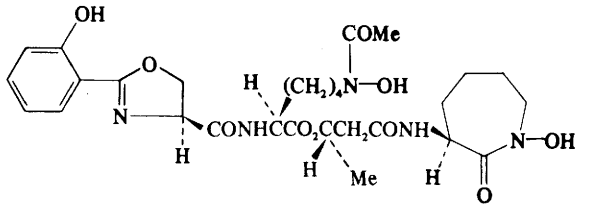
succinic anhydride and the resulting azlactone is cleaved with HI–Red P in refluxing acetic acid has been reported.

Laevulinic acid, converted successively into the 3-bromo derivative, whose oxime was subjected to Beckmann rearrangement, provides a starting material for the synthesis of aspartic acid.[92a] DL-β-Carboxyaspartic acid is accessible through alkylation of the Schiff base Z-N=$CHCO_2Me$ with the anion of di-t-butyl malonate.[92b]

Among a variety of more complex natural amino acids and their derivatives synthesized recently there are to be found examples already mentioned earlier in this chapter. Hypusine has been synthesized[93] through condensation of *N*-benzyloxycarbonyl-L-lysine benzyl ester with the chiral isoxazolidine (6), reduction of the resulting imine with $NaBH_4$, and hydrogenolysis over Pd–C. A synthesis of 9-epihypusine was achieved through the same route using the appropriate isoxazolidine.[93] Another lysine derivative, mycobactin S2 (7),



(6)

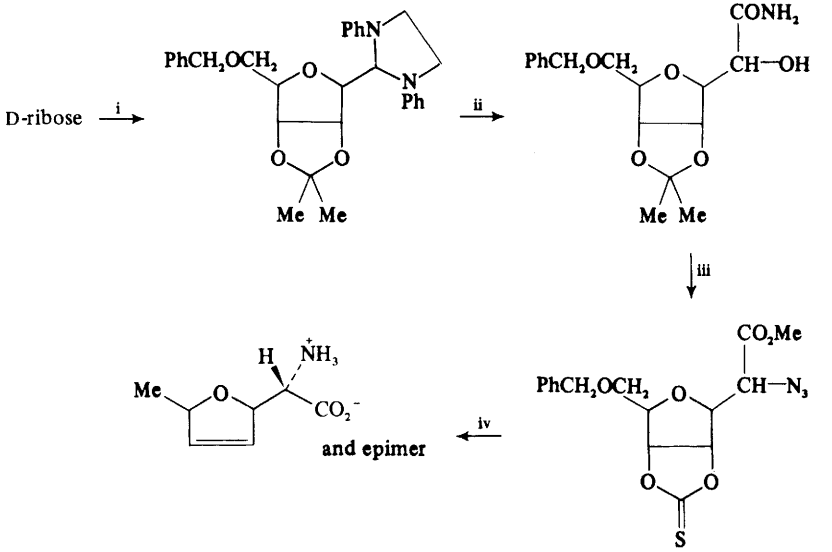


(7)

which differs from mycobactin S in that it carries a methyl group in place of a long hydrocarbon chain, has been synthesized in a route that is unexceptional as far as the amino acid chemistry is concerned.[94] Similarly routine chemistry is involved in the conversion of (*S*)-(−)-phenylalanine into the new derivative (−)-anabellamide [(*S*, *S*)-$PhCONHCH(CH_2Ph)CO_2CH_2CH(NHBz)CH_2Ph$] isolated from *Anaphalis subumbellata*,[95] in the synthesis of all four diastereoisomers of histopine from L-histidine and pyruvic acid,[96] and in the synthesis of the proline analogue of nicotianamine (see p. 24 of this volume, also Vol. 4, p. 4) from L-proline ethyl ester and a protected aspartic semi-aldehyde.[97] More is involved in a synthesis of a diastereoisomer of the antibiotic (+)-furanomycin from D-ribose (Scheme 3), which incidentally confirms the necessity of revision of the stereochemistry assigned to the antibiotic.[98]

Total synthesis of α-allokainic acid has been achieved through a multi-stage procedure (Scheme 4),[99] and reactions of kainic acid itself (modification of the isopropenyl grouping through Pd-catalysed arylation, $CMe=CH_2 \rightarrow CMe=CHAr$,

[92] (*a*) U. R. Joshi and P. A. Limaye, *Indian J. Chem., Sect. B*, 1982, **21**, 1122; (*b*) D. H. Rich and M. K. Dhaon, *Tetrahedron Lett.*, 1983, **24**, 1671.
[93] C. M. Tice and B. Ganem, *J. Org. Chem.*, 1983, **48**, 5048.
[94] P. J. Maurer and M. J. Miller, *J. Am. Chem. Soc.*, 1983, **105**, 240.
[95] S. K. Talapatra, M. K. Pal, A. K. Mallik, and B. Talapatra, *J. Nat. Prod.*, 1983, **46**, 140.
[96] Y. Kitajima, M. Waki, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 3870.
[97] J. Faust, A. Priess, K. Schreiber, and H. Ripperger, *Tetrahedron*, 1983, **39**, 1593.
[98] M. J. Robins and J. M. R. Parker, *Can. J. Chem.*, 1983, **61**, 317.
[99] G. A. Kraus and J. O. Nagy, *Tetrahedron Lett.*, 1983, **24**, 3427.



Reagents: i, known sequence; ii, modified Strecker-type synthesis; iii, hydrolysis, $CSCl_2$; iv, Corey–Winter reaction

Scheme 3

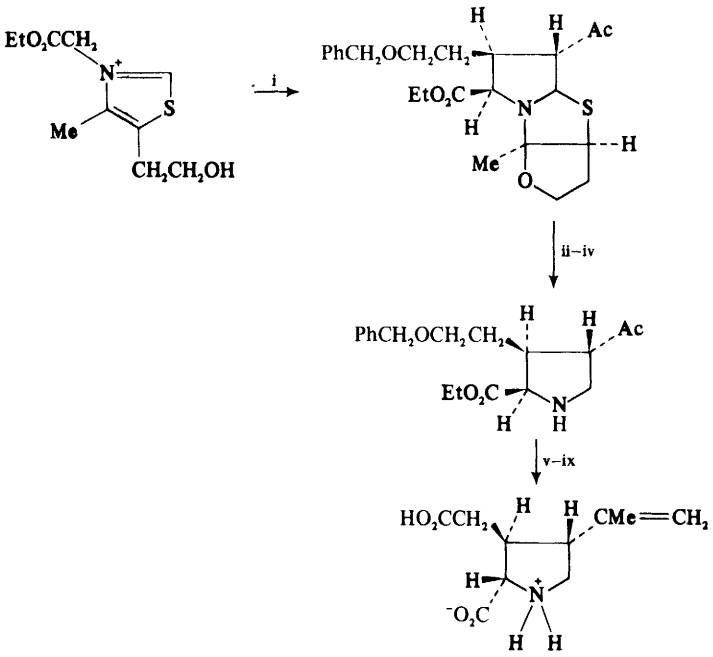
and allylic substitution through alkylation of the π-allylpalladium complex with carbanions from t-butyl acetoacetate or $MeCOCH_2SPh$) have been described.[100]

Synthesis of β- and Higher Homologous Natural Amino Acids. – 4-Amino-alkanoic acids feature in the crop of papers eligible for citation here, including 3-methylstatine analogues $BocNHCHRC(OH)MeCH_2CO_2Et$, prepared from Boc-α-amino acids by treatment with MeLi followed by $MeCO_2Et$ and BuLi,[101] and the use of the corresponding material (Boc-D-alanine) in a related procedure for the synthesis of (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylpentanoic acid, a constituent of bleomycin.[102] A homologue, (*S*)-4-amino-3-hydroxybutyric acid, has been synthesized from D-arabinose *via* conventional stages through a route easily adapted to yield (*S*)-carnitine.[103]

Detoxinine (8) has been synthesized in racemic form through the sequence shown in Scheme 5.[104]

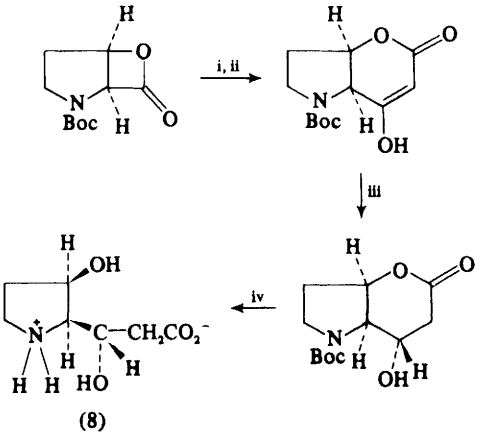
α-Alkyl Analogues of Natural α-Amino Acids. – Preceding sections include descriptions of syntheses of αα-disubstituted α-amino acids through standard methods. Further examples with enhanced interest describe syntheses of α-methyl-

[100] G. A. Conway, J. S. Park, L. Maggiora, M. P. Mertes, N. Galton, and E. K. Michaelis, *J. Med. Chem.*, 1984, **27**, 52.
[101] M. Kawai, A. S. Boparai, M. S. Bernatowicz, and D. H. Rich, *J. Org. Chem.*, 1983, **48**, 1876.
[102] R. M. Di Pardo and M. G. Bock, *Tetrahedron Lett.*, 1983, **24**, 4805.
[103] K. Bock, I. Lundt, and C. Pederson, *Acta Chem. Scand., Ser. B*, 1983, **B37**, 341.
[104] J. Hausler, *Liebigs Ann. Chem.*, 1983, 982.



Reagents: i, $PhCH_2O(CH_2)_2CH{=}CHAc$, Et_3N, MeCN; ii, Bu_3SnH; iii, HCl–EtOH; iv, Boc_2O; v, H_2–Pd/C; vi, Jones oxidation then $Ph_3P{=}CH_2$ on methyl ester; vii, KOH–MeOH; viii, TFA; ix, aq. NaOH, 1 h, 155 °C

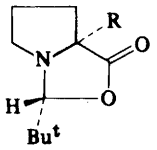
Scheme 4



Reagents: i, Meldrum's acid (2,2-dimethyl-1,3-dioxan-4,6-dione) with 4-(dimethylamino)-pyridine; ii, heat; iii, amine–borane reduction; iv, Boc cleavage and lactone ring-opening

Scheme 5

and -carboxymethyl-ornithine and -arginine,[105] through alkylation of di-lithiated 3-aminopiperidin-2-one Schiff bases followed by cleavage with HCl,[105] and α-alkyl-threonines and -cysteines produced similarly from derived oxazolines.[106] Oxazolidinones (9; R = H), formed by condensing L-proline with pivalic aldehyde, undergo alkylation by electrophiles after lithiation with conservation of the configuration of the proline chiral centre.[107]

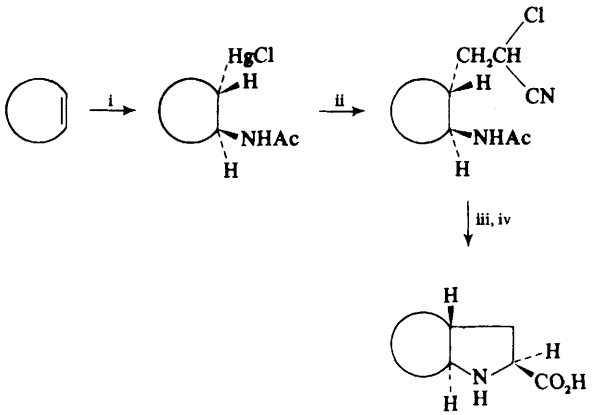


(9)

Synthesis of Other Aliphatic, Alicyclic, and Saturated Heterocyclic Amino Acids. — The Proceedings of the 17th European Peptide Symposium include a number of points of interest of amino acid chemistry, a representative example being a synthesis of 'L-neopentylglycine' (2-amino-4,4-dimethylpentanoic acid) through the sequence $Bu^tCH{=}C(CN)CO_2H \rightarrow Bu^tCH_2CH(CN)CO_2H \rightarrow Bu^tCH_2CH(CONH_2)CO_2H$ followed by Hofmann rearrangement and resolution, using brucine, of the *N*-formyl derivative.[108] Alkylation of ethyl acetamidocyanoacetate with 3-bromomethylcyclohexene gives β-(3-cyclohexenyl)alanine, after alkaline hydrolysis and decarboxylation, which on epoxidation gives a starting material for the synthesis of tetaine analogues.[109] Other straightforward syntheses lead to the 2-aminosuberic acid derivative $BzlOCO(CH_2)_5CH(NHAc)CO_2Et$ through alkylation of benzylideneglycine ethyl ester[110] and to 6-diazo-5-oxo-L-norleucine from methyl *N*-trifluoroacetyl γ-benzyl-L-glutamate through conventional elaboration of the side-chain carboxy group[111] (the foregoing derivative was also elaborated further through its side-chain carboxy group to yield the corresponding oxiranyl ketone[110]).

Proline derivatives and analogues continue to present synthetic challenge, and all the examples representing this year's literature are fully characterized in stereochemical terms. Full details of the synthesis of enantiomers of 5-oxaproline (Vol. 14, p. 11) have been reported.[112] Favorskii ring contraction, a method used before for the synthesis of proline analogues but without specification of stereochemical aspects, is illustrated in the conversion (10) → (11).[113] The same workers

[105] C. G. Unson and B. W. Erickson, *Int. J. Pept. Protein Res.*, 1983, **22**, 50.
[106] D. Seebach and J. D. Aebi, *Tetrahedron Lett.*, 1983, **24**, 3311; D. Seebach and T. Weber, *ibid.*, p. 3315.
[107] D. Seebach, M. Boes, R. Naef, and W. B. Schweizer, *J. Am. Chem. Soc.*, 1983, **105**, 5390.
[108] J. Pospisek and K. Blaha in 'Proceedings of the 17th European Peptide Symposium', ed. K. Blaha and P. Malon, de Gruyter, Berlin, 1983, p. 333.
[109] M. Smulkowski, M. Dzieduszycka, and E. Borowski, *Pol. J. Chem.*, 1982, **56**, 699.
[110] D. H. Rich, J. Singh, and J. H. Gardner, *J. Org. Chem.*, 1983, **48**, 432.
[111] G. R. Pettit and P. S. Nelson, *J. Org. Chem.*, 1983, **48**, 741.
[112] A. Vasella, R. Voeffray, J. Pless, and R. Huguenin, *Helv. Chim. Acta*, 1983, **66**, 1241.
[113] R. Henning and H. Urbach, *Tetrahedron Lett.*, 1983, **24**, 5339.



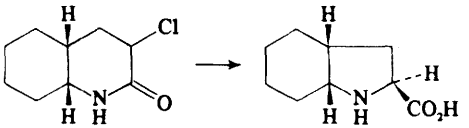
Reagents: i, MeCN, $Hg(NO_3)_2$, NaCl; ii, $CH_2{=}C(CN)Cl$, $NaBH_4$; iii, NaH–DMF; iv, refluxing 5M HCl

Scheme 6

have described a stereospecific synthesis of 4,5-disubstituted proline analogues starting from an alkene (Scheme 6),[114] verifying the stereochemistry through *X*-ray crystal analysis.[115]

Catalytic hydrogenation of 2-ethylpyrrol-1-ine-5-carboxylic acid gave *cis*-5-ethyl-DL-proline, while $NaBH_4$ reduction gave the *cis–trans* mixture from which the *trans* isomer could be isolated *via* *N*-tosyl derivatives.[116] Conventional resolution (employing tartaric acid) completed the task, to provide monomers from which the optically active poly(amino acid)s were prepared.

Higher homologous amino acids are represented in the synthesis of *cis*- and *trans*-2-aminocycloalkaneacetic acids as γ-aminobutyric acid analogues[117] and homo-β-proline analogues.[118]



(10) (11)

[114] R. Henning and H. Urbach, *Tetrahedron Lett.*, 1983, **24**, 5343.
[115] R. Henning, H. Urbach, and E. F. Paulus, *Tetrahedron Lett.*, 1983, **24**, 5347.
[116] W. W. Y. Wang, C. G. Overberger, and C. M. Venkatachalam, *J. Polym. Sci., Polym. Chem. Ed.*, 1983, **21**, 1643.
[117] P. D. Kennewell, S. S. Matharu, J. B. Taylor, R. Westwood, and P. G. Sammes, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2553.
[118] I. M. Labouta, P. Jaconsen, P. Thorbeck, P. Krogsgaard-Larsen, and H. Hjeds, *Acta Chem. Scand., Ser. B*, 1982, **36**, 669.
[119] T. Shono, Y. Matsumura, and K. Inoue, *J. Chem. Soc., Chem. Commun.*, 1983, 1169.

Synthesis of α-Alkoxy-α-amino Acids. – Anodic oxidation of *N,N*-dimethoxycarbonyl-αω-diamino acids in methanol containing tetramethylammonium toluene-*p*-sulphonate gives the ω-methoxy derivative, which can be cyclized *in situ* by adding conc. H_2SO_4 to give saturated heterocyclic amino acids.[119] However, use of NaCl as electrolyte in the same system gives α-methoxy analogues which on cyclization give unsaturated heterocyclic amino acids.[119]

Synthesis of Halogenoalkyl Amino Acids. – All the halogens are represented, in the preparation of 4,4-difluoro-L-proline from the 4-oxoproline and Et_2NSF_3,[120] in the formation of β-fluoro-α-amino acids from cyano- and aminocarbonylaziridines and HF followed by acid hydrolysis,[121] in the formation of *erythro*- or *threo*-4-chloro- or -4-bromo-glutamic acids from the corresponding hydroxyglutamic acids and *N*-methoxycarbonylphthalimide and the phosphorus pentahalide,[122] and in an improved procedure for the synthesis of monoiodohistidine in 57% yield from iodine and the amino acid.[123]

Synthesis of Aliphatic Amino Acids Containing Side-chain Hydroxy Groups. – A convenient synthesis of (*S*)-isoserine from L-malic acid monoamide involves Hofmann rearrangement using $(CF_3CO)_2IPh$ in Ac_2O-pyridine.[124]

Synthesis of Aliphatic Amino Acids Containing Unsaturated Side Chains. – Most space is taken, as usual in this section, by 'dehydroamino acids' (*i.e.* 2-amino-2-alkenoic acids). These can be prepared by dehydration of *N*-Z- or -Boc-2-amino-3-hydroxyalkanoic acids with *N,N'*-dicarbonyldi-imidazole,[125] by dehydration of *N*-acyl-*N*-hydroxy-2-aminoalkanoic acids with $TsCl–Et_3N$,[126] and by $Hg(OAc)_2$ oxidation of vinylogous amino isocyanides followed by treatment with a primary amine.[127]

N-Acetyldehydroalanine may be arylated on the methylene group using an aryl halide with $Pd(OAc)_2$–Ph_3P or $PdCl_2(Ph_3P)_2$, to give homologous *Z*-dehydroamino acids.[128]

βγ-Unsaturated α-alkyl-α-amino acids have been prepared[129] by deconjugative base-catalysed alkylation of methyl α-isocyanoacrylates followed by hydrolysis.

Alkylation of diethyl benzamidomalonate with $Me_3SiC{\equiv}CCH_2Br$ and NaH gives *N*-benzoyl propargylglycine on decarboxylation, which with *N*-iodosuccinimide gives the lactone of *Z*-2-benzamido-4-hydroxy-5-iodopent-4-enoic acid.[130]

[120] J. R. Sufrin, T. M. Balasubramanian, C. M. Vora, and G. R. Marshall, *Int. J. Pept. Protein Res.*, 1982, **20**, 438.
[121] A. I. Ayi and R. Guedj, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2045.
[122] I. M. Kocheva, L. V. Alekseeva, and V. P. Krasnov, *Zh. Org. Khim.*, 1983, **19**, 283.
[123] M. Miyashita, Y. Seyama, K. Kaji, and S. Yamashita, *Chem. Pharm. Bull.*, 1983, **31**, 386.
[124] R. Andruszkiewicz, A. Czerwinski, and J. Grzybowska, *Synthesis*, 1983, 31.
[125] R. Andruszkiewicz and A. Czerwinski, *Synthesis*, 1982, 968.
[126] T. Kolasa, *Synthesis*, 1983, 539.
[127] C. Herdeis and A. Dimmerling, *Arch. Pharm.*, 1984, **317**, 86.
[128] M. Cutolo, V. Fiandanese, F. Naso, and O. Sciacovelli, *Tetrahedron Lett.*, 1983, **24**, 4603.
[129] K. Nunami, M. Suzuki, and N. Yoneda, *Chem. Pharm. Bull.*, 1982, **30**, 4015.
[130] M. J. Sofia, P. K. Chakravarty, and J. A. Katzenellenbogen, *J. Org. Chem.*, 1983, **48**, 3318.

Corresponding phenyl- and benzyl-substituted benzamidomalonate esters give 2-phenyl and 2-benzyl analogues of these unsaturated α-amino acids.[130]

Synthesis of Aromatic and Heteroaromatic Amino Acids. – (3,4-Methylenedioxyphenyl) acetone, NH_3, $CHCl_3$, and a phase-transfer catalyst are said[131] to yield the corresponding Dopa derivative and its amide, from which by conventional resolution and acid hydrolysis L-(−)-α-methylDopa was obtained.[131] Forphenicinol (3-hydroxy-4-hydroxymethylphenyl glycine) is equally easily accessible through Strecker synthesis from the appropriately protected benzaldehyde, resolvable as its *N*-acetyl derivative using aminoacylase.[132] Related amino acids as synthetic targets have included 3′-aryloxytyrosines,[133] 3′-(*N*-4-acetoxy-1,4-benzoxazin-3-onyl)tyrosines,[134] 'cyclopropylphenylalanine' (1-amino-2-arylcyclopropane-1-carboxylic acid),[135] and a variety of 4′-substituted phenylalanines, where the substituent is one of a number of sulphur functional groups formed by elaboration of a $-SO_2Cl$ placed there by reaction of the amino acid with $ClSO_3H$.[136]

The Strecker synthesis has served for the preparation of 3-(1-tetralinyl)- and 3-(5,6,7,8-tetrahydro-5-quinolyl)-alanine,[137] and other standard routes, or modifications of them, have been used for synthesis of 'phosphotryptophan' (from a formylindole and trimethylphosphonoacetate),[138] 2-alkylthio-*N*-hydroxytryptophans [from indoles and $O{=}NC(=CH_2)CO_2Et$],[139] and other tryptophans through Fischer indole synthesis applied to α-methoxy-*N*-benzylpyrrolidines (prepared by electrochemical α-methoxylation – see also ref. 119).[140]

Syntheses of heteroaromatic amino acids using amino acids as starting materials are of the more obvious types (nitration of tryptophan to give the 6-nitro derivative, followed by a variety of modifications to the nitro function,[141] N^{τ}-hydroxyalkylhistidines from protected histidines by reaction with alkyl halides[142]) or of more subtle types (conversion of α-benzyl *N*-benzyloxycarbonyl-D-aspartate to corresponding benzimidazoles using substituted phenylenediamines[143]).

Synthesis of Amino Acids Containing Sulphur. – Many papers dealing with modified cysteines have appeared. β-Chloro-D-alanine has been converted into a range of *S*-substituted D-cysteines through reactions with corresponding thiols

[131] Y. Shi, H. Zhu, Y. Jiang, X. Sun, and H. Hu, *Nanjing Daxue Xuebao, Ziran Kexue*, 1982, 853 (*Chem. Abstr.*, 1983, **98**, 161 140).
[132] H. Morishima, J. Yoshizawa, R. Ushijima, T. Takeuchi, and H. Umezawa, *J. Antibiot.*, 1982, **35**, 1500.
[133] T. Inoue, K. Naitoh, S. Kosemura, I. Umezawa, T. Sonobe, N. Serizawa, N. Mori, and H. Itokawa, *Heterocycles*, 1983, **20**, 397.
[134] T. Ishizaki, Y. Hashimoto, K. Shudo, and T. Okamoto, *Heterocycles*, 1983, **20**, 1481.
[135] H. Kimura and C. H. Stammer, *J. Org. Chem.*, 1983, **48**, 2440; M. Suzuki, S. Kumar, and C. H. Stammer, *ibid.*, p. 4769.
[136] E. Escher, M. Bernier, and P. Parent, *Helv. Chim. Acta*, 1983, **66**, 1355.
[137] E. Reimann and W. Dammertz, *Arch. Pharm.*, 1983, **316**, 297.
[138] S. F. Chen, S. O. Kumar, and M. Tishler, *Tetrahedron Lett.*, 1983, **24**, 5461.
[139] R. Plate, H. C. J. Ottenheijm, and R. J. F. Nivard, *J. Org. Chem.*, 1984, **49**, 540.
[140] T. Shono, Y. Matsumura, and T. Kanazawa, *Tetrahedron Lett.*, 1983, **14**, 1259.
[141] L. L. Melhacb and N. J. Leonard, *J. Org. Chem.*, 1983, **48**, 5130.
[142] J. B. Campbell, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1213.
[143] J. J. Nestor, B. L. Horner, T. L. Ho, G. H. Jones, G. I. McRae, and B. H. Vickery, *J. Med. Chem.*, 1984, **27**, 320.

mediated by an enzyme from *Pseudomonas putida*.[144] Arylation of cysteine by Dopa through oxidation with H_2O_2 in the presence of an iron–EDTA complex gives mixtures of cysteinylDopas,[145] while alkylation using chiral aziridinecarboxylate esters gives lanthionines,[146] *threo*-(—)-*S*-(2-carboxypropyl)-L-cysteine from (*S*)-(—)-$HSCH_2CHMeCO_2Me$,[147] 3-methyl-D-cysteine (a constituent of nisin),[148] and *threo*-3,3′-dimethylcysteine.[149]

Methionine has been converted into *S*-t-butyl homocysteine, exploiting sulphonium salt chemistry; t-butylation followed by equilibration with nucleophiles gave the derivative suitable for use in peptide synthesis as a protected homocysteine.[150] Procedures for the isolation of *S*-adenosyl-L-methionine from baker's yeast have been described,[151] and synthesis of *S*-adenosyl-DL-penicillamine is included in a range of new 5′-substituted *S*-adenosylhomocysteine analogues.[152] Diastereoisomers of L-methionine-*S*,*R*-sulphoximine can be separated after *N*-phthaloylation and cyclization to the corresponding tetrahydro-1,2-thiazin-3-ones by fractional crystallization or chromatography.[153]

2-[(1*S*, 3*S*)-1-Amino-3-carboxy-3-hydroxypropyl]thiazole-4-carboxylic acid, 'fragment D' of the antibiotic nosiheptide, has been synthesized in a multi-step procedure starting with a 4,6-benzylidene-D-2-azido-2,3-dideoxyhexose carrying the necessary chiral centres.[154]

Synthesis of Phosphorus-containing Amino Acids. — (±)-Phosphinothricin has been prepared through alkylation of benzylideneglycine ethyl ester with MeP(O)(OMe)$CH{=}CH_2$ followed by hydrolysis in 6M hydrochloric acid.[155] Synthesis of 'phosphotryptophan' has been mentioned earlier in this chapter.[138]

Amino Acids Synthesized for the First Time. — In addition to many amino acids in this category mentioned elsewhere in this chapter, N^{ϵ}-[(2-chloroethyl)nitrosocarbamoyl]-L-lysine,[156] Dopa analogues in which the phenolic hydroxy groups are replaced by acidic nitrogen functional moieties[157] or linked through $-CH_2-$

[144] T. Nagasawa, H. Hosono, H. Ohkishi, Y. Tani, and H. Yamada, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 809.
[145] S. Ito, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 365.
[146] K. Nakajima, H. Oda, and K. Okawa, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 520.
[147] R. J. Parry and M. V. Naidu, *Tetrahedron Lett.*, 1983, **24**, 1133.
[148] T. Wakamiya, K. Fukase, K. Shimbo, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1559; T. Wakamiya, K. Shimbo, T. Shiba, K. Nakajima, M. Neya, and K. Okawa, *ibid.*, 1982, **55**, 3878.
[149] K. Nakajima and K. Okawa, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1565.
[150] G. Chassaing, S. Lavielle, and A. Marquet, *J. Org. Chem.*, 1983, **48**, 1757.
[151] S. V. Petrenko, *Khim.-Farm. Zh.*, 1983, **17**, 330.
[152] D. Shire, P. Blanchard, A. Raies, F. Lawrence, M. Robert-Gero, and E. Lederer, *Nucleosides Nucleotides*, 1983, **2**, 21.
[153] Y. Sugiyama and F. C. Wedler, *Tetrahedron Lett.*, 1983, **24**, 1471.
[154] M. Iwakawa, Y. Kobayashi, S. Ikuta, and J. Yoshimura, *Chem. Lett.*, 1982, 1975.
[155] N. Minowa, S. Fukatu, T. Niida, M. Takada, and K. Sato, *Tetrahedron Lett.*, 1983, **24**, 2391.
[156] J. C. Kim and I. S. Cho, *Yakhak Hoechi*, 1983, **27**, 177 (*Chem. Abstr.*, 1983, **99**, 176 244).
[157] N. Zenker, C. N. Talaty, P. S. Callery, J. Wright, L. S. Hubbard, and E. M. Johnson, *J. Heterocycl. Chem.*, 1983, **20**, 435; H. Schmidhammer and K. Hohenlohe-Oehringen, *Sci. Pharm.*, 1983, **51**, 8.

$(CH_2OCH_2)_nCH_2-$,[158] and DL-thyroxine *p*-hydroxyphenyl ether[159] have been described.

Synthesis of Labelled Amino Acids. – Conference proceedings often provide an economical overview of the current status of a narrow field of study, and the increasing interest in amino acids labelled with short-lived isotopes is represented this year.[160] Only sample citations, synthesis of ^{11}C-carboxyl-labelled and ^{13}N-labelled glutamic acid[160] and synthesis of other ^{11}C-carboxyl-labelled amino acids,[161] are given here. Positron emission from ^{11}C-labelled amino acids offers new possibilities in discovering the fate of amino acids metabolized at different sites *in vivo*, providing the main spur to establishing efficient syntheses that can be effected very rapidly; synthesis of L-[1-^{11}C]leucine[162] provides another example of rapid Strecker synthesis and D-amino acid oxidase resolution within 30–40 min from H^{11}CN.

A crop of papers,[163–172] mostly from laboratories whose work has featured in this section in recent volumes, describe syntheses of protein amino acids variously labelled with carbon, hydrogen, and nitrogen isotopes. An extensive account[163] describes syntheses of chiral-methyl valines, whose very high specific radioactivity renders them well suited for biosynthetic studies. The other labelled amino acids described in these papers are (3*RS*)-β-leucine-[2,3-^{3}H],[164] (2*S*,4*S*)- and (2*R*,4*S*)-leucine-[5-^{13}C] from (2*RS*,3*S*)-valine-[4-^{13}C] *via* (3*S*)-isovaleric acid-[4-^{13}C],[165] (2*RS*)-leucine-[1,2-$^{13}C_2$] from $Me_2CHCH_2{}^{13}CHO$,[165] β-hydroxyvaline-[4-^{13}C] and β-hydroxyvaline-[4,4,4-$^2H_3$],[166] L-glutamic acid labelled stereospecifically at C-3 with ^{2}H and non-stereospecifically at C-4 with ^{3}H,[167] L-serine stereospecifically labelled at C-3 with ^{2}H,[168] aspartic and glutamic acids-[1-^{13}C] and glutamic acid-[3-^{13}C],[169] branched-chain amino acids-[1-^{13}C,^{15}N],[170] glutamic acid-[1-^{13}C,^{15}N] and related amino acids,[171] and (*RS*)-glycine-[α-^{2}H].[172]

The other main approach to labelled amino acids employs the amino acids themselves or an amino acid from which the target molecule is accessible. Factors determining the distribution achieved in catalysed ^{3}H–^{1}H exchange with $^3H_2$ have

[158] M. Berthet and E. Sonveaux, *J. Chem. Soc., Chem. Commun.*, 1983, 10.
[159] K. G. Boldt and G. A. Brine, *Org. Prep. Proced. Int.*, 1983, **15**, 137.
[160] M. B. Cohen, L. Spotter, C. C. Chang, and N. S. MacDonald in 'Proceedings of 3rd World Congress, Nuclear Medicine, Biological Advances', ed. C. Raynaud, Pergamon, Oxford, 1983, Vol. 1, p. 632.
[161] Q. Sun, S. Chen, Y. Ye, H. Bao, Y. Li, F. Fan, C. Gu, L. Zhang, and Z. Zhan in ref. 160, p. 650.
[162] J. R. Barrio, R. E. Keen, J. R. Ropchan, N. S. MacDonald, F. J. Baumgartner, H. C. Padgett, and M. E. Phelps, *J. Nucl. Med.*, 1983, **24**, 515.
[163] D. H. G. Crout, M. Lutstorf, and P. J. Morgan, *Tetrahedron*, 1983, **39**, 3469.
[164] D. J. Aberhart and H. J. Lin, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 611.
[165] D. J. Aberhart and B. H. Weiller, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 663.
[166] D. J. Aberhart, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 605.
[167] S. J. Field and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2387.
[168] D. Gani and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2393.
[169] U. Fotader and D. Cowburn, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 1003.
[170] S. S. Yuan, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 173.
[171] O. Bouloussa and P. Dizabo, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 991.
[172] R. H. White, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 787.

been discussed.[173] Alkylation of L-homocysteine with C^3H_3I,[174] formation of L-asparagine-[U-^{13}C] and the corresponding L-aspartic acid,[175] and H_2O_2–Fe^{2+} oxidation of proline-[U-^{14}C] to give the corresponding *trans*-3-hydroxyproline[176] provide routine examples of synthesis of labelled protein amino acids, as do hydrogenation of dehydrophenylalanyl-D-alanine dioxopiperazine with $^2H_2$ followed by hydrolysis,[177] conversion of glycine-[2-^{3}H] into the corresponding creatine,[178] and conversion of L-canaline into L-[guanidino-oxy-^{14}C] canavanine by reaction with *O*-[^{14}C-methyl]isourea.[179]

^{15}N-Labelled L-lysine and L-glutamic acid have been prepared by fermentation (*Brevibacterium flavum*,[180] *Brevibacterium lactofermentum*,[181] respectively) with $^{15}NH_4^+$ salts as nitrogen source, while ^{13}N-labelled leucine and valine have been prepared from the corresponding keto acids, $^{13}NH_3$, and immobilized glutamate dehydrogenase.[182]

Halogen isotopes are discussed in a representative citation from conference proceedings describing ^{18}F-labelled difluoroaminoalkyl analogues of amino acids (*e.g.* conversion of ornithine *via* its lactam into 2-amino-[5-^{18}F]-difluoro-aminopentanoic acid by reaction with $^{18}F_2$ in CF_3CO_2H[183]) and introduction of the same isotope into proline by treatment of *N*-tosyl hydroxy-L-proline methyl ester with $Et_4N^{18}F$.[184] Radio-bromination and -iodination of aromatic amino acids can be effected using the halogen with Chloramine-T or Dichloramine-T.[185]

Resolution of Amino Acids. – The main topic areas (diastereoisomer salt formation, the use of enzymes, chromatographic resolutions, and preferential crystallization) are fully represented in the recent literature. The topic has been reviewed recently.[186]

N-Acylated amino acids have been resolved through diastereoisomeric salt formation (Boc-DL-phenylglycine with phenylethylamine,[187] formyl-DL-neopentylglycine with brucine,[108] *N*-acetyl-3-(3,4-methylenedioxyphenyl)-2-methylalanine with quinine[131]), and *cis*-5-ethyl-DL-proline has been resolved using

[173] B. V. Petrenik, Yu. A. Zolotarev, and N. F. Myasoedov, *Bioorg. Khim.*, 1983, **9**, 1021.
[174] Z. Guo and G. Tang, *He Huaxue Yu Fangshe Huaxue*, 1983, **5**, 255 (*Chem. Abstr.*, 1984, **100**, 34 794).
[175] T. H. Lam, R. Mermet-Bouvier, S. Fermandjian, and P. Fromageot, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 143.
[176] S. G. Ramawamy, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 233.
[177] K. Tanimura, T. Kato, M. Waki, and N. Izumiya, *Tetrahedron Lett.*, 1983, **24**, 3737.
[178] M. M. Daly and I. Lalezari, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 377.
[179] G. A. Rosenthal, K. R. Downum, and J. E. Mattler, *Anal. Biochem.*, 1983, **133**, 277.
[180] C. S. Irving, C. L. Cooney, L. T. Brown, D. Gold, J. Gordon, and P. D. Klein, *Anal. Biochem.*, 1983, **131**, 93.
[181] Z. E. Kahana and A. Lapidot, *Anal. Biochem.*, 1983, **132**, 160.
[182] K. R. Barrio, F. J. Baumgartner, E. Henze, M. R. Stauber, J. E. Egbert, N. S. MacDonald, H. L. Schelbert, M. E. Phelps, and F. T. Liu, *J. Nucl. Med.*, 1983, **24**, 937.
[183] D. Jewett and R. Ehrenkaufer in 'Proceedings of the International Symposium on Synthetic Applications of Isotopically Labelled Compounds', ed. W. P. Duncan and A. B. Susan, Elsevier, Amsterdam, 1983, p. 205.
[184] M. van der Ley, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 453.
[185] G. S. Petzold, *Ber. Kernforschungsanlage Juelich*, 1982 (*Chem. Abstr.*, 1983, **99**, 5984).
[186] T. Kitahara, *Kagaku Kogaku*, 1983, **47**, 277.
[187] A. W. Lipkowski, *Pol. J. Chem.*, 1981, **55**, 1725.

L-tartaric acid.[116] The related diastereoisomeric derivative approach is illustrated in a resolution of DL-cysteine by conversion into diastereoisomeric thiazolidines through reaction with D-(+)-galactose (or any one of a number of aldohexoses or aldopentoses).[188] Related procedures have been used for the resolution of DL-2-amino-4-(aryloxy)butanoic acids[189] and 'cyclopropylphenylalanine' (1-amino-2-phenylcyclopropanecarboxylic acid).[135]

Enzymic methods, such as preferential catalysis of hydrolysis of an *N*-acetyl-L-amino acid in the presence of its enantiomer by aminoacylase, have been used for the resolution of forphenicinol,[132] phenylalanine,[190] and other examples mentioned elsewhere in this chapter. The same principle applied to DL-5-substituted hydantoins using hydantoinase from *Pseudomonas striata*[191] can be particularly satisfactorily conducted on a large scale.

Chromatographic resolution continues along the established paths, but with more discernible attempts to reason out the optimum systems for the purpose. Silica-gel chromatography of *N*-4-nitrobenzoyl-DL-amino acid isopropyl esters with a chiral mobile phase (typically chloroform–n-hexane, *N*-acetyl-L-valine) achieves enantiomeric discrimination through formation of diastereoisomeric chelate-like solvates through intermolecular hydrogen bonds with the D- and L-enantiomers of the amino acid derivative.[192] It is possible that earlier-described silica-gel systems assumed to involve the chiral additive as part of the stationary phase are in fact functioning in the manner now suggested (or *vice versa*). L-Histidine bonded to silica gel[193] and a similar L-valine t-butylamide-bonded stationary phase[194] are described as effective in the resolution of acetylamino acid methyl esters, and further results are reported for L-hydroxyproline bonded to polystyrene and complexed with Cu^{2+} ions for ligand-exchange chromatographic resolutions of DL-amino acids.[195,196]

Preferential crystallization of one enantiomer from supersaturated solutions seeded with one enantiomer is effective for DL-phenylglycine sulphate[197] and for DL-threonine and DL-serine *m*-xylenesulphonates,[198] for salts of *N*-acetyl-DL-phenylalanine with secondary amines,[199] and for ammonium *N*-benzoyl-DL-alaninate.[200] A new approach exploiting the same principle is illustrated in the

[188] J. Martens and K. Drauz, *Liebigs Ann. Chem.*, 1983, 2073.
[189] B. Witczuk, M. Strube, H. Jeschkeit, and G. Kupryszewski, *Pol. J. Chem.*, 1981, **55**, 1511.
[190] T. Kitahara and S. Asai, *Agric. Biol. Chem.*, 1983, **47**, 991.
[191] S. Takahashi, *Hakko Kogaku Kaishi*, 1983, **61**, 139 (*Chem. Abstr.*, 1983, **99** 51 815).
[192] A. Dobashi and S. Hara, *J. Chromatogr.*, 1983, **267**, 11; *Anal. Chem.*, 1983, **55**, 1805; *Tetrahedron Lett.*, 1983, **24**, 1509.
[193] N. Watanabe, *J. Chromatogr.*, 1983, **260**, 75.
[194] X. Xu, Q. Tang, and R. Wang, *Huazue Xuebao*, 1983, **41**, 262.
[195] Yu. A. Zolotarev and N. F. Myasoedov, *J. Chromatogr.*, 1983, **264**, 377.
[196] V. A. Shirokov, V. A. Tsyryapkin, L. V. Nedospasova, A. A. Kurganov, and V. A. Davankov, *Bioorg. Khim.*, 1983, **9**, 878.
[197] T. Shiraiwa, Y. Ohmichi, K. Iwafuji, K. Fujimoto, and H. Kurokawa, *Nippon Kagaku Kaishi*, 1983, 1070 (*Chem. Abstr.*, 1984, **100**, 7090).
[198] O. Otsuki, *Kemikaru Enjiniyaringu*, 1983, **28**, 148 (*Chem. Abstr.*, 1983, **99**, 122 841).
[199] T. Shiraiwa, S. Taniguchi, A. Ikawa, K. Iwafuji, and H. Kurikawa, *Nippon Kagaku Kaishi*, 1983, 1189 (*Chem. Abstr.*, 1984, **100**, 7097).
[200] T. Shiraiwa, Y. Ohmichi, and H. Kurokawa, *Nippon Kagaku Kaishi*, 1983, 1102 (*Chem. Abstr.*, 1983, **99**, 158 807).

resolution of DL-aspartic acid by seeding a solution with the copper(II) complex of an optically active amino acid, which competitively inhibits the crystallization of one of the enantiomers.[201]

4 Physical and Stereochemical Studies of Amino Acids

Crystal Structures of Amino Acids and Their Derivatives. – Following a listing of amino acids and derivatives other than peptides that have been subjected tc *X*-ray crystal analysis, points of general interest are discussed.

Recent studies have involved *N*,*N*-dimethylglycine hydrochloride,[202] the *N*-chloroacetyl derivative of (–)-isovaline (thus shown to possess the *R*-configuration),[203] L-aspartic acid,[204] DL-glutamic acid monohydrate,[205] L-homoserine,[206] DL-α-methyl-*m*-tyrosine,[207] Boc-L-proline,[208] N^{α}-Boc-N^{π}-benzyloxymethyl-L-histidine (thus confirming other physical evidence that the imidazole substituent is located on the nitrogen atom closest to the chiral centre),[209] L-arginine acetate,[210] L-lysine acetate,[211] L-ornithine L-aspartate hemihydrate,[212] bis(methyl-L-cysteinato) molybdenum dioxygenate,[213] and cyclo(L-asparagyl-L-asparagyl).[214]

Two strong hydrogen bonds per molecule exist in solid *N*,*N*-dimethylglycine hydrochloride,[202] one ($-N-H\cdots Cl^-$) being perpendicular to the plane of the cation. The form of L-aspartic acid studied[204] unusually carries a neutral α-carboxy group and a deprotonated side-chain carboxy group.

In the crystal structures of amino acids generally, a head-to-tail hydrogen-bonded sequence of amino and carboxylate moieties is almost always seen.[215] The structures revealed for the acetates of L-arginine[210] and L-lysine[211] also show the same feature, whereas the parent amino acids and their salts and complexes do not. The weakly acidic acetic acid component therefore interacts with the most basic (side-chain) grouping in these amino acids in the solid state, allowing the head-to-tail arrangement, as preferred by the zwitterionic moiety, to be

[201] K. Harada and N. Fujii, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 653.
[202] B. D. Santarsiero and R. E. Marsh, *J. Crystallogr. Spectrosc. Res.*, 1983, **13**, 245.
[203] R. Bosch, H. Brückner, G. Jung, and W. Winter, *Tetrahedron*, 1982, **38**, 3579.
[204] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 176.
[205] Z. Ciunik and T. Glowiak, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1983, **39**, 1271.
[206] K. K. Chacko, S. Swaminathan, and K. R. Veena, *Cryst. Struct. Commun.*, 1982, **11**, 2057.
[207] K. A. Satyshur and S. T. Rao, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1983, **C39**, 1672.
[208] K. Takahashi and M. Obayashi, *Kinki Daigaku Rikogakubu Kenkyu Hokoku*, 1983, 57 (*Chem. Abstr.*, 1983, **99**, 140 347).
[209] T. Brown, J. H. Jones, and J. D. Wallis, *J. Chem. Soc., Perkin Trans. 1*, 1982, 3045.
[210] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **21**, 223.
[211] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 617.
[212] D. M. Salunke and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 154.
[213] I. Buchanan, M. Minelli, M. T. Ashby, T. J. King, J. H. Enemark, and C. D. Garner, *Inorg. Chem.*, 1984, **23**, 495.
[214] C. Howes. N. W. Alcock, B. T. Golding, and R. W. McCabe, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2287.
[215] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 129.

adopted. The head-to-tail arrangement seen in the crystal structure of L-ornithine L-aspartate is the first example for a mixed amino acid salt.

Nuclear Magnetic Resonance Spectrometry. – ^{1}H and ^{13}C n.m.r. studies are now frequently performed and reported jointly, and this is reflected in the structure of this section. Heavier-element n.m.r. studies of amino acids and their derivatives are reviewed in order of increasing atomic number.

Familiar themes are represented in ^{1}H n.m.r. titration studies of a series of imidazoles (including L-histidine and its isomeric *N*-methyl derivatives) yielding microscopic p*K* values of the different imidazole tautomers[216] and in conformational interpretations [*N*-acetyl-α-aminoisobutyric *N*-methylamide adopts a conformation lacking hydrogen bonds;[217] the flexible thiazolidine ring in 3-thiaproline hydantoin preferentially adopts an envelope conformation (C-4 as the 'flap');[218] the side chains of methionine and norleucine derivatives in MeOH adopt closely similar conformations, while *S*-methylcysteine and norvaline derivatives differ in this respect;[219] five- and seven-membered hydrogen-bonded ring structures are adopted by *N*-acetyl-L-phenylalanine *p*-acetyl- and *p*-valeryl-anilides in non-polar solvents[220]]. ^{13}C n.m.r. studies continue to give crucial information in biosynthetic studies using strategically labelled amino acids, showing for example that, while the *m*,*m'*-dihydroxyphenylglycine and *p*-methyl-*m*,*m'*-dihydroxyphenylglycine moieties of ristocetin can be derived from acetate units, the *p*-hydroxyphenylglycine moiety is derived from tyrosine.[221] ^{1}H and ^{13}C n.m.r. data for tryptophan and derivatives have been collected,[222] mainly to offer help in structure elucidations of indole alkaloids.

Because much of the foregoing material is now routine (perhaps an idiosyncratic definition by the Reporter, pressed to select from a broad range of possible citations in certain areas as a result of space limitations imposed on him), more detailed discussion is reserved for more sophisticated instrumental possibilities. Intramolecular separations of hydrogen atoms within the 20 most common amino acids have been determined by n.O.e. studies,[223] while for L-histidine longitudinal and transverse ^{1}H relaxation rates for aqueous solutions give information about molecular motion and conformational changes as a function of time.[224] Other solution studies include ^{1}H, ^{13}C, and INDO studies of L-tryptophan and related compounds[225] and ^{13}C longitudinal relation times (T_1) and

[216] M. Tanokura, *Biochim. Biophys. Acta*, 1983, **742**, 576.
[217] Y. Paterson, E. R. Stimson, D. J. Evans, S. J. Leach, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1982, **20**, 468.
[218] F. A. M. Borremans, M. Budesinsky, R. E. A. Callens, and M. J. O. Anteunis, *Org. Magn. Reson.*, 1983, **21**, 328.
[219] B. Picur and I. Z. Siemion, *Org. Magn. Reson.*, 1983, **21**, 271.
[220] D. Petkov, P. Ivanov, and I. Stoineva, *Biopolymers*, 1983, **22**, 1489.
[221] S. J. Hammond, D. H. Williams, and R. C. Nielsen, *J. Chem. Soc., Chem. Commun.*, 1983, 116.
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[225] S. Biagini, A. Lai, M. Monduzzi, and G. Saba, *J. Chem. Soc., Faraday Trans. 2*, 1983, **79**, 491.

n.O.e. for uniformly ^{13}C-enriched glycine and aspartic acid.[226] Solid-state studies are tending to give information more remote from that which the organic chemist and the analytical chemist need to have to hand, such as ^{2}H n.m.r. spin-lattice relaxation data giving energies for re-orientation of methyl groups in polycrystalline, strategically ^{2}H-labelled amino acids.[227] Similar ^{13}C n.m.r. studies have been described,[228, 229] also ^{13}C chemical-shielding tensors[230] and ^{14}N quadrupole tensors in single crystals of L-threonine and L-serine monohydrate, respectively.[231]

^{15}N n.m.r. can yield information on the barriers to isomerization about the $C-N_2$ bond in guanidinium and guanidino groups in L-arginine; although interconversion is rapid on the n.m.r. time-scale in water–dimethyl sulphoxide through the temperature range −52 to 25 °C, the energy barrier is significantly greater for the guanidinium form.[232] The data can be used to indicate that in this environment approximately one-third of the guanidino form is present as the $RN{=}C(NH_2)_2$ tautomer.[232] The effect of pH on natural-abundance ^{15}N n.m.r. chemical shifts of lysine and ε-(hydroxymethyl)lysine has been interpreted to yield pK_0 values of the nitrogen functional groups.[233]

^{19}F n.m.r. has been used to follow the reaction between *erythro*- or *threo*-β-fluoroaspartic acid and pyridoxal 5′-phosphate, providing stereochemical information for the reaction products through $^{3}J(HF)$ data.[234] Another fundamental application of ^{19}F n.m.r., the pH dependence of chemical shifts for mono-, di-, and tri-fluoro-α-methylalanines and their methyl esters, has been described.[235]

Optical Rotatory Dispersion and Circular Dichroism. — As in many aspects of n.m.r. spectrometry, these techniques are largely free from unexplored fundamental areas deserving publication in the primary literature. Considerable interest remains, however, in applications of these techniques in systems in which an amino acid creates an asymmetric environment for an achiral molecule, for example the positive c.d. induced in catechol crown ethers in the presence of D-glutamic acid hydrochloride.[236] This behaviour can be exploited for the assignment of absolute configuration to an enantiomer of an amino acid. An example of the use of chromophore-substituted amino acids[237] in the manner featured in this

[226] H. Nery, D. Canet, F. Toma, and S. Fermandjian, *J. Am. Chem. Soc.*, 1983, **105**, 1482.
[227] L. S. Batchelder, C. H. Niu, and D. A. Torchia, *J. Am. Chem. Soc.*, 1983, **105**, 2228.
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[230] N. Janes, S. Ganapathy, and E. Oldfield, *J. Magn. Reson.*, 1983, **54**, 111.
[231] A. Naito, S. Ganapathy, P. Raghunathan, and C. A. McDowell, *J. Chem. Phys.*, 1983, **79**, 4173.
[232] K. Kanamori and J. D. Roberts, *J. Am. Chem. Soc.*, 1983, **105**, 4698.
[233] N. Naulet, D. Tome, and G. J. Martin, *Org. Magn. Reson.*, 1983, **21**, 564.
[234] M. C. Salon, S. Hamman, and C. G. Beguin, *Org. Magn. Reson.*, 1983, **21**, 265.
[235] J. S. Taylor and C. Deutsch, *Biophys. J.*, 1983, **43**, 261.
[236] J. Hu, Z. Guo, and Z. Gao, *Fenzi Kexue Yu Huaxue Yanjiu*, 1983, **3**, 63 (*Chem. Abstr.*, 1983, **99**, 140 331).
[237] N. A. Voskova, V. V. Romanov, G. A. Korshunova, and Yu. P. Shvachkin in 'Proceedings of the 3rd U.S.S.R.–F.R.G. Symposium on the Chemistry of Peptides and Proteins', ed. W. Voelter, E. Wünsch, and Yu. A. Ovchinnikov, de Gruyter, Berlin, 1982, p. 373.

section in every past volume involves the measurement of both c.d. and u.v. spectra near 340 nm of *o*-phthaldialdehyde–mercaptoethanol condensation products formed with mixtures of enantiomers of an amino acid to assess the enantiomeric composition of the sample.

Instrumental advances allowing penetration to shorter wavelengths lead to fuller c.d. spectral information for amino acid derivatives than that obtained in earlier years. Thus, *N*-acetyl-L-tyrosinamide shows positive Cotton effects centred near 230 and 200 nm and negative Cotton effects centred near 215 and 183 nm.[238] Access to vibrational c.d. data is also becoming easier, a recent example establishing a methine C—H stretching chirality rule. L-α-Amino acids in ^2H_2O give vibrational c.d. spectra with a strong positive bias in the CH stretching region.[239] Since the intensity bias seems sensitive to the presence and strength of intramolecular hydrogen bonds, further useful information about fundamental structural features should be expected from this technique.

Mass Spectrometry. – The biological literature is the main source of what is now routine analytical use of this technique, usually in conjunction with g.l.c. Representative papers in this area describe g.l.c.–m.s. estimation of [guanidino-^{15}N]arginine as its tetra-trifluoroacetyl derivative[240] and 1-aminocyclopropane-1-carboxylic acid content of Douglas pine by g.l.c.–m.s. after h.p.l.c. purification of its 2,4-dinitrophenyl derivative.[241] Even the food-analysis literature, however, now contains descriptions of the newer techniques in mass spectrometry, *e.g.* analysis of free amino acids by positive and negative chemical ionization[242] (in contrast to electron-impact m.s., chemical-ionization m.s. yields spectra carrying prominent intact-molecule ion peaks, revealing relatively little fragmentation in the case of amino acid samples). Laser-desorption mass spectrometry of valine similarly yields spectra revealing the minimal fragmentation ion associated with some desorption ionization techniques, valine being characterized by a prominent peak at m/z 118 (*i.e.* M + 1).[243] Secondary-ion emission by bombardment of amino acid films on metal layers results in formation of M + 1 positive ions through proton transfer between adjacent molecules, glycine[244] and phenylalanine[245,246] having been studied in pioneering studies involving irradiation of

[238] E. J. Welsh, S. A. Frangou, E. R. Morris, D. A. Rees, and S. I. Chavin, *Biopolymers*, 1983, **22**, 821.

[239] L. A. Nafie, M. R. Oboodi, and T. B. Freedman, *J. Am. Chem. Soc.*, 1983, **105**, 7449.

[240] I. Nissim, M. Yudkoff, T. Terwilliger, and S. Segal, *Anal. Biochem.*, 1983, **131**, 75.

[241] R. A. Savidge, G. M. C. Mutumba, J. K. Heald, and P. F. Wareing, *Plant Physiol.*, 1983, **71**, 434.

[242] D. Fraisse, F. Maquin, J. C. Tabet, and H. Chaveron in 'Proceedings of the 1st European Conference on Food Chemistry: Recent Developments in Food Analysis', ed. W. Balters, P. Czedik-Eysenburg, and W. Pfannhauser, Verlag Chemie, Weinheim, 1982, p. 190.

[243] D. M. Hercules, C. D. Parker, K. Balasanmugam, and S. K. Viswanadhan, *Springer Ser. Chem. Phys.*, 1983, **25** (*Ion Formation in Organic Solids*), 222.

[244] W. Lange, M. Jirikowsky, D. Holtkamp, and A. Benninghoven, *Springer Ser. Chem. Phys.*, 1982, **19** (*Secondary Ion Spectrometry*), 416.

[245] W. Sichtermann, *Springer Ser. Chem. Phys.*, 1983, **25** (*Ion Formation in Organic Solids*), 132.

[246] W. Guthier, O. Becker, S. Della Negra, W. Knippelberg, Y. Le Beyec, U. Weikert, K. Wien, P. Wieser, and R. Wurster, *Int. J. Mass Spectrom. Ion Phys.*, 1983, **53**, 185.

the films by ^{16}N and ^{238}U ions of fixed energies. Formation of layers[247] involves ultra-high-vacuum deposition by a molecular-beam technique, and since the 2000 Å films yield ions whose characteristics depend strongly on the chemical nature of the metal matrix on which they are deposited it is not likely that these ionization techniques will enter rapidly into routine service.

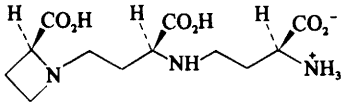
Other Physical Studies. – This section deals with certain other spectroscopic studies, but mainly with a wide range of physical data collected for amino acids and their derivatives.

Raman techniques assist the study of the phase transition that occurs at 80 °C in crystalline L-leucine, supporting i.r. data in identifying a conformational change within the amino acid side chain as being responsible.[248] A conventional i.r.–Raman study of α-glycine and its perdeuteriated derivative at 300 and 85 K has been reported.[249] *N*-Acetylamino acid esters in CCl_4 show additional peaks in the C–O stretching region (1000–1300 cm^{-1}) assigned to Fermi-resonance absorption.[250] Thymine–DL-tryptophan aggregates formed by lyophilization are in the form of stacked pyrimidine–indole moieties.[251]

Helium photoelectron spectra of glycine, sarcosine, and glycine methyl ester and of representative *N*-acetyl amino acids have been interpreted in terms of localized molecular orbitals.[252]

p*K* values 6.97, 9.13, and 9.75 and stability constants for complexes with a range of divalent metal ions have been determined for nicotianamine, a likely phytosiderophoric amino acid (12).[253] The same measurements have been performed for L-Dopa[254] and for *N*-(*m*-substituted phenyl)glycines,[255] and stability constants have been measured for ternary complexes between adenine or cytosine, tryptophan or histidine, and a divalent metal cation.[256] Partial molar volumes at infinite dilution,[257] viscosities,[258] heats of solution,[259] osmotic and activity coefficients,[260] and interfacial properties[261] of amino acids and their

[247] W. Lange, D. Holtkamp, M. Jirikowsky, and A. Benninghoven, *Springer Ser. Chem. Phys.*, 1983, **25** (*Ion Formation in Organic Solids*), 124.
[248] D. Bougeard, *Ber. Bunsenges. Phys. Chem.*, 1983, **87**, 279.
[249] B. Andrews, B. H. Torrie, and B. M. Powell, *Biophys. J.*, 1983, **41**, 293.
[250] V. Slet, *Eesti NSV Tead. Akad. Toim., Keem.*, 1983, **32**, 134 (*Chem. Abstr.*, 1983, **99**, 105 656).
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[252] P. H. Cannington and N. S. Ham, *J. Electron Spectrosc. Relat. Phenom.*, 1983, **32**, 139.
[253] I. Benes, K. Schreiber, H. Ripperger, and A. Kircheiss, *Experientia*, 1983, **39**, 261.
[254] T. Kiss and A. Gergely, *Magy. Kem. Foly.*, 1983, **89**, 81.
[255] R. Chen and H. Lin, *Huaxue Xuebao*, 1983, **41**, 87 (*Chem. Abstr.*, 1983, **98**, 198 683).
[256] M. M. T. Khan and S. Satyanarayana, *Indian J. Chem., Sect. A*, 1983, **22**, 584.
[257] F. Shahidi, *J. Solution Chem.*, 1983, **12**, 295; D. V. Jahagirdar and S. U. Pankanti, *Indian J. Chem., Sect. A*, 1983, **22**, 195; A. K. Mishra, K. P. Prasad, and J. C. Ahluwalia, *Biopolymers*, 1983, **22**, 2397; A. K. Mishra and J. C. Ahluwalia, *J. Phys. Chem.*, 1984, **88**, 86.
[258] S. J. Kim, Y. J. Oh, K. S. Choi, and Y. K. Shin, *Bull. Korean Chem. Soc.*, 1983, **4**, 284; J. S. Sandhu, U. Kashyap, and R. K. Wadi, *J. Indian Chem. Soc.*, 1983, **60**, 192; M. M. Bhattacharyya and M. Sengupta, *Z. Phys. Chem.*, 1982, **133**, 79.
[259] M. Matsumoto and K. Amaya, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2521.
[260] O. D. Bonner, *Physiol. Chem. Phys.*, 1982, **14**, 539.
[261] K. Ogino, H. Yamauchi, and T. Shibayama, *Yukugaku*, 1982, **31**, 1009 (*Chem. Abstr.*, 1983, **98**, 179 843).



(12)

salts have been reported. The last-mentioned study involves the effect of amino acids as solutes on the surface and interfacial tensions between aqueous and non-polar (dodecane or oleic acid) phases;[261] a similar partition study[262] in which distribution coefficients are measured for *N*-acetyl amino acid amides of the twenty protein amino acids and for the extremely lipophilic amino acids carboranylalanine and adamantylalanine yields hydrophobic parameters for the side chains involved. Transport of amino acids across membranes is another related topic of study, moving closer to biological systems; transport of L-cysteine across brush border membranes of rat renal cortex provides a specific example, the movement being inhibited by L-alanine and L-phenylalanine but not by L-glutamic acid or basic L-amino acids.[263] Penetration of L-lysine hydrochloride into lecithin monolayers[264] is another example of 'artificial transport' of glycine or alanine through complexation with the macrocyclic poly(imine) 1,4,7,10-tetra-*N*-benzyl-1,4,7,10-tetra-azadodecane and Ni^{2+} ions, also of phenylalanine or leucine with the same tetra-amine with Cu^{2+} ions;[265] similar results were obtained using a crown-ether analogue.[266] This is an important laboratory model for a plausible *in vivo* mechanism for conversion of zwitterionic amino acids into lipophilic complexes.

Thermochemical studies range from the relatively routine (enthalpies of solution of L-aspartic and glutamic acids in water at low concentrations[267]) to the more adventurous investigation of energetics of protonation of *N*-acetylalanine methyl ester.[268] In comparison with data for *N*,*N*-dimethylacetamide, the amino acid derivative shows higher proton affinity and negative entropy of protonation, indicating the existence of intramolecular hydrogen bonding in the protonated amino acid derivative. Since the internal hydrogen bond decreases the availability of the proton for intermolecular hydrogen bonding, the attachment energy of water to the protonated amino acid derivative is less than that for the protonated dimethylacetamide. Continuation of studies of gas-phase acidity and basicity of glycine sees an extension to various methyl homologues;[269] unlike the crystal-state situation, glycine exists in the non-zwitterionic form in the gas phase.[269]

Easily visible differences in the morphology of centrosymmetric crystals precipitated in the presence of one enantiomer of a chiral amino acid, compared with their appearance when precipitated in the presence of the other enantiomer,

[262] J. L. Fauchere and V. Pliska, *Eur. J. Med. Chem., Chim. Ther.*, 1983, **18**, 369.
[263] B. Stieger, G. Stange, J. Biber, and H. Murer, *J. Membr. Biol.*, 1983, **73**, 25.
[264] M. Nakagaki and E. Okamura, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1607.
[265] H. Tsukube, *J. Chem. Soc., Perkin Trans. 1*, 1983, 29.
[266] H. Tsukube, *J. Membr. Sci.*, 1983, **14**, 155.
[267] X. Ni and R. Hu, *Huaxue Xuebao*, 1983, **41**, 41 (*Chem. Abstr.*, 1983, **98**, 198 682).
[268] M. Meot-Ner, *J. Am. Chem. Soc.*, 1984, **106**, 278.
[269] M. J. Locke and R. T. McIver, *J. Am. Chem. Soc.*, 1983, **105**, 4226.

permit the assignment of absolute configuration to an amino acid[270] (see also Vol. 14, p. 18). The structural requirements of four host molecules (which must be chiral or prochiral) and additives have been outlined; glycine was used in this study as a suitable prochiral host.[270]

Molecular-orbital Calculations. – Major themes over recent years are continued, with papers considering hydrogen bonding and other interactions between amino acids and simple ions or solvent molecules, conformational aspects, and calculations intended to arrive at fundamental data that may be compared with those determined experimentally.

The amide oxygen atom of an *N*-acylated amino acid is calculated[271] to be a stronger proton acceptor than a carboxyl oxygen atom. A summary paper[272] updates the data for geometrical parameters, non-bonded interactions, and hydrogen-bond interactions to take account of experimental information of the last 10 years. Calculations are better able to fit experimental i.r. and Raman spectral parameters and to predict side-chain behaviour of amino acid residues in peptides and proteins more realistically when the revised data are used. Other MO calculations in this general area include hydration geometries of protonated lysine, arginine, and glutamate and aspartate ions,[273] of the 20 common amino acids,[274] of the complex ions $[Cu(Ile)]^+$, $[Cu(Thr)]^+$, and $[Cu(Thr)_2H_{-2}]^{2-}$,[275] of glycine and its zwitterion,[276] and of γ-aminobutyric acid in the presence of Mg^{2+} and Ca^{2+} ions.[277] The relative energies of the three intramolecularly hydrogen-bonded glycine conformers (two with bifurcated hydrogen bonds from $-NH_2$ to carbonyl oxygen and to $-OH$ oxygen, respectively, and one with a hydrogen bond from OH to N) have been computed, indicating the first-mentioned structure to be preferred.[278] Dipole moments, charge density, and ionization potentials have been calculated for neutral and zwitterionic forms of glycine, alanine, serine, cysteine, and selenocysteine.[279]

Factors influencing intermolecular interactions that MO calculations can illuminate include hydrophobicity parameters[280] and amino acid–nucleotide stacking interactions;[281] tryptophan emerges as the residue most able, amongst the aromatic and heteroaromatic amino acids, to form stable interactions of this type. Conformational information arises from calculations concerning *N*-acetyl

[270] I. Weissbuch, L. Addadi, Z. Berkovitch-Yellin, E. Gati, S. Weinstein, M. Lahav, and L. Leiserowitz, *J. Am. Chem. Soc.*, 1983, **105**, 6615.
[271] Z. Berkovitch-Yellin, S. Ariel, and L. Leisorowitz, *J. Am. Chem. Soc.*, 1983, **105**, 765.
[272] G. Nemethy, M. S. Pottle, and H. A. Scheraga, *J. Phys. Chem.*, 1983, **87**, 1883.
[273] G. Ranghino, E. Clementi, and S. Romano, *Biopolymers*, 1983, **22**, 1449.
[274] S. Fraga, *Theochem.*, 1983, **11**, 251.
[275] N. Raos and V. Simeon, *J. Inorg. Biochem.*, 1983, **18**, 133.
[276] E. E. David and C. W. David, *J. Chem. Phys.*, 1983, **78**, 1459.
[277] I. O. Blake, A. Les, and S. Rybak, *J. Theor. Biol.*, 1983, **104**, 571.
[278] S. Millefiori and A. Millefiori, *Theochem*, 1983, **8**, 391.
[279] A. E. Smolyar, A. R. Abramov, A. A. Guliev, O. N. Narimanbekov, and T. N. Shakhtakhtinskii, *Azerb. Khim. Zh.*, 1982, 20.
[280] M. Charton and B. I. Charton, *J. Theor. Biol.*, 1982, **99**, 629.
[281] N. V. Kumar and G. Govil in 'Conformation in Biology', ed. R. Srinivasan and R. H. Sarma, Adenine Press, Guilderland, New York, 1983, p. 313.

alanine methylamide,[282] the glycine analogue and near relatives,[283] and the corresponding derivatives of the 20 protein amino acids, together with γ-aminobutyric acid,[284] using up-dated interatomic energies and residue geometries (*cf.* ref. 272). The disulphide linkage in L-cysteine and in simpler disulphides is at its minimum-energy conformation at a dihedral angle close to 90°.[285]

Calculations allowing band assignments in vibrational spectra of cysteine, serine, and β-chloroalanine have been performed, including consideration of seven isotopically substituted analogues.[286]

5 Chemical Studies of Amino Acids

Racemization. – Papers describing mechanistic aspects of amino acid racemization argue in favour[287] of a two-step process with abstraction of the methine proton being rate-determining. There is also evidence[288] that racemization of L-[1-^{2}H]-alanine proceeds with significant internal return of the proton when catalysed by tyrosine phenol lyase and the amino acid racemase from *Pseudomonas striata*, supporting the idea that a single base is involved in the process. Alkaline racemization of *Z*-γ-thiapipecolic acid pentachlorophenyl ester is some seven times faster than that of the γ-thiaproline analogue[289] and some four times faster than that of the pipecolic acid analogue. This confirms the rate-enhancing effect of a nearby sulphur atom[290] and also reveals that finer aspects of the geometrical relationship between the sulphur atom and the chiral centre are important since the rate enhancement associated with the sulphur atom in a five-membered ring is substantially greater than that for an acyclic analogue.[289]

Practical exploitation of amino acid racemization follows familiar lines, with further studies of racemization of α-amino acids in hot AcOH[291] in the presence of 0.05 mol of an aldehyde showing that complete dissolution of the amino acid is not required; the racemate can simply be filtered off after an appropriate time has elapsed. The combination of the same general principle (use of pyridoxal 5′-phosphate with a DL-amino acid ester in neutral aqueous solution) with catalysis of hydrolysis by an esterase allows continuous production of an L-amino acid to be achieved.[292]

[282] J. N. Scarsdale, C. van Alsenoy, V. J. Klimkowski, L. Schaefer, and F. A. Momany, *J. Am. Chem. Soc.*, 1983, **105**, 3438; C. Tosi, *Nuovo Cimento Soc. Ital. Fis. D*, 1983, **2**, 15.

[283] P. S. Stern, M. Chorev, M. Goodman, and A. T. Hagler, *Biopolymers*, 1983, **22**, 1885, 1901.

[284] M. Vasquez, G. Nemethy, and H. A. Scheraga, *Macromolecules*, 1983, **16**, 1043.

[285] R. J. Boyd, J. S. Perkyns, and R. Ramani, *Can. J. Chem.*, 1983, **61**, 1082.

[286] H. Susi, D. M. Byler, and W. V. Gerasimowicz, *J. Mol. Struct.*, 1983, **102**, 63.

[287] G. G. Smith and T. Sivakua, *J. Org. Chem.*, 1983, **48**, 627; E. D. Stroud, D. J. Fife, and G. G. Smith, *ibid.*, p. 5368.

[288] S. J. Shen, H. G. Floss, H. Kumagai, H. Yamada, N. Esaki, K. Soda, S. A. Wasserman, and C. Walsh, *J. Chem. Soc., Chem. Commun.*, 1983, 82.

[289] D. P. M. Wante, F. A. M. Borremans, and M. J. O. Anteunis, *Bull. Soc. Chim. Belg.*, 1983, **92**, 49.

[290] M. Barber, J. H. Jones, and M. J. Witty, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2425.

[291] S. Yamada, C. Hongo, R. Yoshioka, and I. Chibatu, *J. Org. Chem.*, 1983, **48**, 843.

[292] M. Pugniere, A. Commeyras, and A. Previero, *Biotechnol. Lett.*, 1983, **5**, 447.

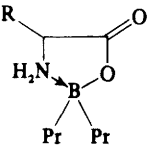
The less accessible literature carries a recent example of the use of amino acid racemization for dating purposes,[293] ages of shells from Sardinia dated by this method agreeing with relative stratigraphy implications.

General Reactions. – Reactions leading to *N*-substituted derivatives for use in synthesis or for analytical purposes are described in accounts of formylation in refluxing HCO_2H–DMF,[294] t-butoxycarbonylation using the stable reagent t-butyl benzotriazol-1-yl carbonate[295] or using di-t-butyl carbonate,[296] tritylation through initial silylation with $AcN(SiMe_3)_2$ followed by reaction with trityl chloride,[297] phthaloylation using *N*-(ethoxycarbonyl)phthalimide,[298] *N*-benzoylethylation through Mannich reactions with amino acid esters, phenyl alkyl ketones, and paraformaldehyde,[299] and formation of *N*-2,2,2-trichloroethylidene Schiff bases by reaction with chloral followed by dehydration of the resulting aminols with $SOCl_2$.[300] 9-Fluorenylmethyl esters, cleavable under mild conditions using a secondary amine, have been prepared from Boc-amino acids using 9-fluorenylmethanol and dicyclohexylcarbodi-imide.[301] Oxazoles formed by reaction of a *Z*-amino acid with benzoin and treatment of the resulting ester with NH_4OAc are readily cleaved by singlet oxygen to yield the corresponding *Z*-amino acid dibenzoylamide.[302]

Other reactions involving amino and carboxy groups (*i.e.* leading to heterocyclic structures) are illustrated in conversions of amino acids into keto acids[303] and into diketones,[304] by refluxing with trifluoroacetic anhydride in the former case and by successive treatment with trifluoroacetic anhydride, Michael addition to t-butyl acrylate, addition of an organo-magnesium or -lithium compound, and cleavage of the resulting oxazolinols with HCO_2H. 2-Trifluoromethyloxazolin-5-ones are the common intermediate in these two processes. Boroxazolidinones (13) are formed from amino acids with tris-(n-propyl)borane in refluxing xylene.[305]

3-Phenylfuran is one of the products formed in the Maillard reaction between glucose and phenylalanine;[306] this long-established general reaction involving amino acids and carbohydrates has been reviewed.[307] Other reactions of almost equally long acquaintance also continue to be represented in the literature,

[293] P. Wanet, F. Leone, A. Ozer, and A. Ulzegha, *Rend. Semin. Fac. Sci. Univ. Cagliari*, 1982, **52**, 159 (*Chem. Abstr.*, 1983, **99**, 174 860).
[294] J. M. Aizpurua and C. Palomo, *Synth. Commun.*, 1983, **13**, 745.
[295] S. Kim and H. Chang, *J. Chem. Soc., Chem. Commun.*, 1983, 1357.
[296] G. Perseo, S. Piani, and R. De Castiglione, *Int. J. Pept. Protein Res.*, 1983, **21**, 227.
[297] A. K. Rabinovich, V. N. Karelskii, E. P. Krysin, A. A. Antonov, M. B. Smirnov, and G. Cipens, *Khim. Prir. Soedin.*, 1983, 216.
[298] C. R. McArthur, P. M. Worster, and A. U. Okon, *Synth. Commun.*, 1983, **13**, 393.
[299] H. Haber and H. G. Henning, *Pharmazie*, 1983, **38**, 509.
[300] G. Giedemann and I. Ugi, *Synthesis*, 1983, 788.
[301] H. Kessler and R. Siegmeier, *Tetrahedron Lett.*, 1983, **24**, 281.
[302] H. H. Wasserman and T.-J. Lu, *Tetrahedron Lett.*, 1982, **23**, 3831.
[303] C. Klein, G. Schulz, and W. Steglich, *Liebigs Ann. Chem.*, 1983, 1638.
[304] J. Leyendecker, U. Niewöhner, and W. Steglich, *Tetrahedron Lett.*, 1983, **24**, 2375.
[305] G. H. L. Nefkens and B. Zwanenburg, *Tetrahedron*, 1983, **39**, 2995.
[306] G. Westphal and E. Cieslik, *Nahrung*, 1982, **26**, 765.
[307] G. Vernin, J. Metzger, and T. Obustunov, *Actual. Chem.*, 1983, 7.



(13)

including deamination by nitrous acid. Alanine methyl ester hydrochloride in acetic acid yields chloro-, acetoxy-, and hydroxy-substitution products and methyl acrylate,[308] and homologues yield products of 1,2-shifts of the β-substituent in yielding β-fluoroalkanoates as well as α-fluoro-substitution products in reaction with $NaNO_2$/HF–pyridine[309] (see also Vol. 14, p. 27). Oxidative degradation of amino acids by Chloramine-T in both acid and alkaline media has been thoroughly re-examined from a mechanistic point of view;[310] in acid media slow *N*-monochlorination of the amino acid is followed by a rapid formation of the *N,N*-dichloro compound, which undergoes elimination of HCl and subsequent hydrolysis. Alternatively, Cl_2 or H_2OCl^+ formed in water by disproportionation of Chloramine-T reacts with the amino acid as just described; in alkaline media the reagents can include HOCl and its anion.[310] The chemistry involved in these pathways, *i.e.* the concerted fragmentation of *N*-chloroamino-acid anions to give imines (which hydrolyse rapidly to amines and carbonyl compounds) and CO_2, proceeds at rates highly dependent upon the amino acid structure (Gly < sarcosine < Thr < Ala < Pro < α-aminoisobutyric acid < 1-aminocyclohexanecarboxylic acid).[311]

Other decarboxylation reactions are featured in the reaction of amino acids with 2,4,6-triphenylpyrylium salts to give corresponding *N*-alkylpyridinium salts[312] and in a moderate-yield pyrolysis (145 °C) of an *N*-acyl dehydroamino acid in the presence of copper and heteroaromatic base.[313]

The classical oxidative degradation of amino acids alluded to in the preceding paragraph has many variations already committed to the literature, and the routine exploitation of these employing metal ions as oxidants continues;[314] use of a diacyl peroxide for the purpose converts an amino acid ester into either an *N*-acyl derivative or an *N*-acyloxy analogue, depending on steric effects

[308] Z. A. Malik, *Pak. J. Sci. Ind. Res.*, 1982, **25**, 5.

[309] S. Hamman and C. G. Beguin, *Tetrahedron Lett.*, 1983, **24**, 57.

[310] B. T. Gowda and D. S. Mahadevappa, *J. Chem. Soc., Perkin Trans. 2*, 1983, 323; D. S. Mahadevappa, M. S. Ahmed, N. M. M. Gowda, and B. T. Gowda, *Int. J. Chem. Kinet.*, 1983, **15**, 775; D. S. Mahadevappa, K. S. Rangappa, N. M. M. Gowda, and B. T. Gowda, *Indian J. Chem., Sect. A*, 1983, **22**, 631.

[311] V. C. Hand, M. P. Snyder, and D. W. Margerum, *J. Am. Chem. Soc.*, 1983, **105**, 4022.

[312] A. R. Katritzky, N. E. Grzeskowiak, N. F. Eweiss, and E. A. Elsherbini, *J. Chem. Soc., Perkin Trans. 1*, 1983, 497.

[313] U. Schmidt and A. Lieberknecht, *Angew. Chem.*, 1983, **95**, 575.

[314] C. M. Ashraf, I. Ahmed, and F. K. N. Lugemwa, *Z. Phys. Chem.*, 1983, **264**, 794; Y. Yoshikawa and K. Yamasaki, *J. Indian Chem. Soc.*, 1982, **59**, 1250; U. D. Mudaliar, V. R. Chourey, R. S. Verma, and V. R. Shastry, *J. Indian Chem. Soc.*, 1983, **60**, 561; K. Behari, N. Saxena, M. Verma, and B. Krishna, *Natl. Acad. Sci. Lett. (India)*, 1982, **5**, 293; R. S. Shukla, R. K. Dwivedi, K. C. Gupta, and K. Behari, *ibid.*, p. 297; S. P. Srivastava and B. B. L. Mathur, *Rev. Roum. Chim.*, 1983, **28**, 27.

imposed by either reactant.[315] A study, non-routine by any standards, involves argon-arc plasma-produced oxidant, probably $HO^{\bullet}$, which effects side-chain oxidation of representative amino acids; for example[316] alanine yields serine and glycine, among other products, in aqueous solutions.

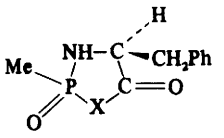
A very considerable emphasis on mechanistic aspects is evident in these studies; aminolysis and hydrolysis studies continue the same theme. Boc-glycine active esters[317] and glycine ethyl ester co-ordinated into mixed copper(II) complexes[318] were the systems on which straightforward structure–reactivity studies were based, while a study of the hydrolysis of *N*-benzyloxycarbonyl-amino acid *p*-nitrophenyl esters in the presence and absence of free amino acids constitutes a more subtle challenge.[319] The high rate seen for the lysine derivative in the absence of an amino acid seems due to intramolecular electrostatic interaction of the positively charged ε-amino group with the negatively charged tetrahedral intermediate, and several other similar conclusions are drawn in this study.[319] General acid catalysis by HCO_3^- of the breakdown of the tetrahedral intermediate that forms during the alkaline hydrolysis of *N*-acetylglycine anilides has been observed.[320]

Enantioselective catalysis is seen in the hydrolysis of amino acid *p*-nitrophenyl esters,[321,322] of *p*-nitrophenyl alkanoates,[323,369] and of histidine methyl ester[324] by poly(acrylamide)s[321] and dodecane-1,10-dioic diamides[323] in which the amide moiety is an L-histidine residue, by micellar *N*-lauroyl- and *N*-palmitoyl-L-histidine and -L-histidyl-L-leucine,[322] and by crosslinked hydrophilic polymer supports bearing L-histidine residues complexed with Ni^{2+} ions.[324] Generally, the greater selectivity is seen with the more hydrophilic carrier molecules (though a brief generalization such as this cannot do justice to the wealth of detail in these studies).

Solvolysis of *N*-[amino(methyl)phosphinyl]-L-phenylalanine esters and amides and their hydroxyphosphinyl and phosphinothioyl analogues involves the heterocyclic intermediate (14).[325]

A variety of functional-group modifications amounting to improvements of known reactions includes conversion of Boc-amino acids into aldehydes *via* *N*-(methoxy)methylamides using $LiAlH_4$,[326] sodium–liquid ammonia reduction

[315] M. Milewska, T. Kolasa, and A. Chimiak, *Pol. J. Chem.*, 1981, **55**, 2215.
[316] K. Harada and M. Takasaki, *Tetrahedron Lett.*, 1983, **24**, 4839.
[317] S. K. Girin and Yu. P. Shvachkin, *Zh. Obshch. Khim.*, 1983, **53**, 2371.
[318] B. Przeczek, H. Langfelderova, and J. Gazo in 'Proceedings of the 9th Conference on Co-ordination Chemistry', p. 349 (*Chem. Abstr.*, 1984, **100**, 7089).
[319] P. Ascenzi, G. Sleiter, and E. Antonini, *Gazz. Chim. Ital.*, 1982, **112**, 307.
[320] D. Petkov and I. Pozharliev, *Izv. Khim.*, 1982, **15**, 247.
[321] I. Cho and J. S. Shin, *Makromol. Chem.*, 1983, **184**, 147.
[322] R. Ueoka, Y. Matsumoto, T. Kikuno, and K. Okada, *J. Mol. Catal.*, 1983, **18**, 267; R. Ueoka and Y. Murakami, *J. Chem. Soc., Perkin Trans. 2*, 1983, 219; see also Y. Ihara, R. Hosako, M. Nango, and N. Kuroki, *J. Chem. Soc., Perkin Trans. 2*, 1983, 5.
[323] M. Kodaka, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2191; see also M. Tanihara and Y. Imanishi, *Polym. J. (Tokyo)*, 1983, **15**, 499.
[324] N. Spassky, M. Reix, M. O. Sepulchre, and J. P. Guette, *Makromol. Chem.*, 1983, **184**, 17.
[325] N. E. Jacobsen and P. A. Bartlett, *J. Am. Chem. Soc.*, 1983, **105**, 1613, 1619.
[326] J. A. Fehrentz and B. Castro, *Synthesis*, 1983, 676.



(14)

of amino acid amides to amino alkanols,[327] and α-methoxylation through anodic oxidation of *N*-acyl amino acid esters in methanol containing an alkali-metal halide or a tetra-alkylammonium halide.[328] Transamination processes in which pyridoxal operates in a membrane is modelled by micellar *N*-lauroyl-pyridoxal Schiff bases, shown to undergo transamination in the absence of metal ions.[329] Corresponding transimination (a deliberately precise term) of pyridoxal 5′-phosphate–ethylamine Schiff base with alanine or aspartic acid has been subjected to kinetic study,[330] indicating a more rapid direct exchange than an alternative 'hydrolysis-followed-by-condensation' pathway. Anchimeric assistance by the 5′-phosphate grouping is also indicated.

An increasing number of synthetic uses is being found for L-amino acids as chiral agents for biassing the asymmetric synthesis of non-peptide natural products. (*S*)-β-Amino alkanols yield Schiff bases with aldehydes from which (*S*,*S*)-1-aryl-2-phenyl-(2-hydroxymethyl)amines are obtained by reaction with a Grignard reagent.[331] An alternative alkylation procedure in which N-protected L-amino acids are converted into corresponding aminoalkyl ketones by reaction of the lithium carboxylate with an organo-lithium or -magnesium reagent has been described.[332] Asymmetric hydrogenation of *N*-pyruvoyl-(*S*)-amino acid esters[333] and phenylglyoxylic analogues[334] using $NaBH_4$ yields (*R*)-lactoyl derivatives in up to 44% diastereoisomeric purity when the reduction is conducted in alcoholic media and (*S*)-tropoyl analogues in equally modest enantiomeric excess when the reduction is carried out in 1% aqueous THF, respectively. Acid hydrolysis of the products yields the optically active α-hydroxy acids. Schiff bases formed between a ketone and an (*S*)-α-amino acid ester give corresponding secondary amines through hydrogenation over Pd–C, which may be cleaved by *N*-chlorination with t-butyl hypochlorite, dehydrochlorination, and hydrolysis of the resulting imine to give the chiral (*S*)-amine derived from the original ketone,[335] optical yields being dependent on both solvent and structure. Asymmetric conjugate addition of *N*-butyl-lithium to *N*-(cinnamoyl)-*S*-proline in the presence of tertiary amines generates a new chiral centre that is predominantly of *R*-configuration, whereas in the absence of tertiary amines the *S*,*S*-diastereo-

[327] I. Schon, T. Szirtes, T. Uberhardt, and A. Csahi, *J. Org. Chem.*, 1983, **48**, 116.
[328] T. Shono, Y. Matsumura, and K. Inoue, *J. Org. Chem.*, 1983, **48**, 1388.
[329] H. Kondo, J. Kikuchi, and J. Sunamoto, *Tetrahedron Lett.*, 1983, **24**, 2403.
[330] S. H. Weng and D. L. Leussing, *J. Am. Chem. Soc.*, 1983, **105**, 4082.
[331] Y. Suzuki and H. Takahashi, *Chem. Pharm. Bull.*, 1983, **31**, 31.
[332] C. G. Knudsen and H. Rapoport, *J. Org. Chem.*, 1983, **48**, 2260.
[333] T. Munegumi and K. Harada, *Chem. Lett.*, 1983, 1225.
[334] K. Soai, H. Hasegawa, K. Komiya, Y. Shigematsu, and A. Ookawa in 'Proceedings of the 20th American Peptide Symposium', p. 81 (*Chem. Abstr.*, 1983, **99**, 105 651).
[335] N. Ikota, K. Achiwa, and S. Yamada, *Chem. Pharm. Bull.*, 1983, **31**, 387.

isomer predominates. Optical yields are low, and apparently unimportant changes in experimental procedure (the order in which the base and BuLi were added) reverse the diastereoselectivity.[336]

Specific Reactions of Natural Amino Acids and Their Derivatives. – Since this section deals mostly with reactions of side chains of common amino acids, it is incidentally an extension of amino acid synthesis in the sense that the products are often novel amino acids. However, the emphasis is on interesting and useful chemistry.

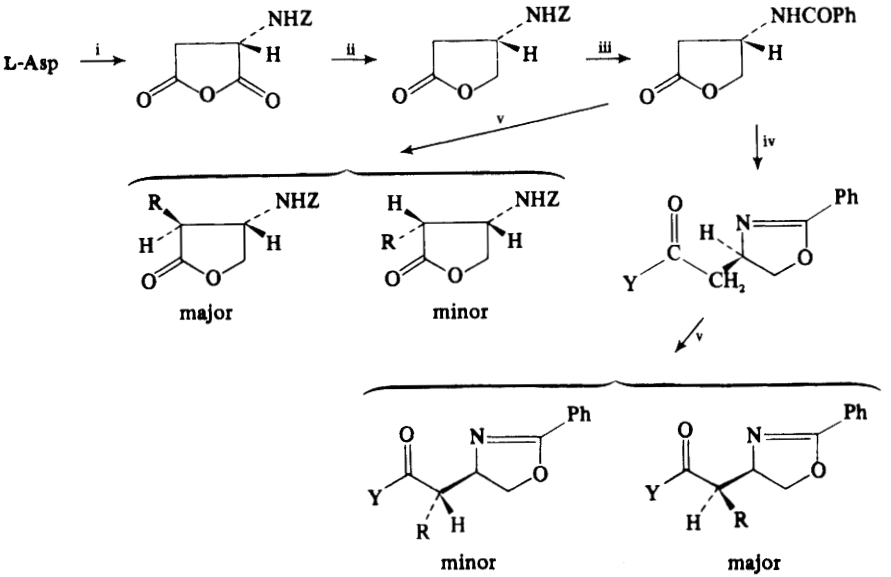
Regioselective chlorination of *N*-benzoylvaline methyl ester with SO_2Cl_2 and benzoyl peroxide gives β- and γ-chloro derivatives. The proportions ($\beta:\gamma = 2:1$) tend to confirm standard textbook information concerning free-radical attack at saturated branched alkyl groups in favour of substitution of the proton at the tertiary carbon atom of the valine side chain.[337] Other studies of aliphatic hydrocarbon side chains include one on condensation of *cis*-bis(glycinato)copper(II) monohydrate with competition between two aldehydes to yield mixtures of β-hydroxy-α-amino acids (preference for the *threo*-isomer of the bulkier product seems demonstrated – acetaldehyde and benzaldehyde give a reaction mixture containing 66% *threo*-β-phenylserine)[338] and a detailed study of the fragmentation of *cis*-dideuterioaminocyclopropanecarboxylic acid into ethylene (loss of stereochemical integrity is observed under *in vivo* conditions, suggesting a free-radical mechanism) with CN^- being a surprising reaction product.[339]

Pyrolysis studies of amino acids have been featured in this section regularly over the years, because of their relevance to food science on the one hand and the study of the organic content of meteorites on the other. L-Glutamic acid and its salts with metals of Groups I and II undergo successive cyclodehydration, dehydration to 5-carboxy-2-pyrrolidone, and further degradation to pyrrole, as shown by thermal-analysis study through the temperature range to 400 °C.[340]

α-Amino-dicarboxylic acids feature in the enantiodivergent alkylation of L-aspartic acid (Scheme 7).[341] The term 'enantiodivergent': L-aspartic acid is the source of alkylation products of its β-carbon atom, and one of the two pathways shown in Scheme 7 favours the *threo*-alkylation product whereas the other favours the *erythro*-isomer.

An efficient synthesis is announced of *N*-2,2,2-trichloro-ethoxycarbonyl-L-aspartyl and -glutamyl α-ethyl and α-benzyl esters from the corresponding Troc-amino acid anhydrides.[342] One of the recently established protein α-amino-tricarboxylic acids, γ-carboxyglutamic acid, has been shown[343] to react readily with aldehydes in what amounts to an intramolecular Mannich reaction to give

[336] K. Soai, A. Ookawa, and Y. Nohara, *Synth. Commun.*, 1983, **13**, 27.
[337] C. J. Easton and N. J. Bowman, *J. Chem. Soc., Chem. Commun.*, 1983, 1193.
[338] P. Sharrock, *Polyhedron*, 1983, **2**, 111.
[339] M. C. Pirrung, *J. Am. Chem. Soc.*, 1983, **105**, 7207.
[340] Z. B. Bakasova, V. I. Kegan, D. A. Abdybaliev, and I. G. Druzhinin, *Izv. Akad. Nauk Kirg. SSR*, 1982, 37.
[341] G. J. McGarvey, R. N. Hiner, Y. Matsubara, and T. Oh, *Tetrahedron Lett.*, 1983, **24**, 2733.
[342] J. F. Carson, *Synthesis*, 1983, 669.
[343] R. Capasso, G. Randazzo, and L. Pecci, *Can. J. Chem.*, 1983, **61**, 2657.



Reagents: i, ZCl then Ac_2O; ii, $NaBH_4$; iii, HBr–AcOH then PhCOCl–py; iv, EtO^- or Me_2NH; v, $LiPr_2N$, RX

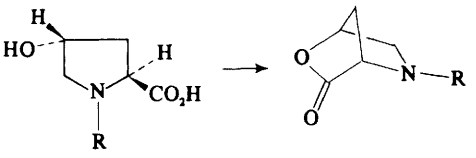
Scheme 7

5-substituted 4,4-dicarboxyprolines. These undergo monodecarboxylation at 100 °C during 30 min.[343]

Side-chain hydroxy groups can participate in elimination, stereospecific conversion of *threo*-β-hydroxy-α-amino acid derivatives into *Z*-dehydroamino acids having been demonstrated using Et_2NSF_3-py.[344] Boc-serine and -threonine can be *O*-acylated under standard conditions by long-chain aliphatic acid chlorides, and elaboration of amino and carboxy groups can then be carried out without disturbing the new side-chain functions.[345] Conversion of *trans*-4-hydroxyprolines (15) into azaoxabicycloheptanes (16) is achievable in one step using $EtO_2CN{=}NCO_2Et$ and Ph_3P;[346] a point of interest is the restriction to rotation imposed on an *N*-acyl group (16; R = MeCO) in its new environment, leading to the existence at ambient temperatures of two rotational isomers.

Several important studies of side-chain thiol, sulphide, and sulphonium groups are represented in the 1983 literature, some of the papers extending earlier studies. Intramolecular transfer of an initially formed *S*-nitroso derivative during the diazotization of L-methionine and *S*-methyl-L-cysteine is suggested by the large rate enhancement seen with these amino acids in comparison with the rate of diazotization of alanine.[347] One enantiomer of *S*-adenosyl-L-methionine

[344] L. Somekh and A. Shanzer, *J. Org. Chem.*, 1983, **48**, 907.
[345] G. V. Marinetti, *Chem. Phys. Lipids*, 1983, **33**, 145.
[346] M. M. Bowers-Nemia and M. M. Joullie, *Heterocycles*, 1983, **20**, 817.
[347] T. A. Meyer and D. L. H. Williams, *J. Chem. Soc., Chem. Commun.*, 1983, 1067.



(15) (16)

(*S*-configuration at sulphur) formed by enzymic synthesis has two pathways open to it in physiological media (pH 7.5, 37 °C), *viz.* inversion at sulphur and nucleophilic cleavage to give homo-L-serine and 5′-deoxy-5′-(methylthio)-adenosine, and factors affecting the balance between these have been studied.[348] Formation of an *S*-centred radical cation through the reaction of methionine with HO• generated from the Ti^{III}–H_2O_2 couple is established as the initiation of oxidative decarboxylation.[349] The α-amino radical formed in this way undergoes reduction considerably more slowly in its protonated form.[350] Copper-catalysed autoxidation of cysteine is accompanied by the formation of H_2O_2,[351] and this is then used for oxidation of further cysteine. The stoicheiometry was established for the ratio of cysteine consumed to H_2O_2 produced as 2:1, and the possibility of a Michaelis–Menten-type mechanism for re-oxidation or oxygenation of copper(I) species produced in the oxidation was also discussed.[351] Straightforward procedures for achieving the reverse process, the reduction of L-cystine, employ electrolysis in aqueous HCl with Pd and graphite electrodes.[352]

Oxidation of selenomethionine by gold(III) in H_2O yields the selenoxide,[353] an observation paralleling the earlier (Vol. 6, p. 32) use of gold(III) for stereospecific oxidation of methionine itself to the sulphoxide. However, selenoxides are considerably more prone to elimination, and the ^1H n.m.r. data on which the assigned chemistry is based may need to be reconsidered to take this into account.

Side-chain aliphatic amino-group chemistry is represented in a clean preparation of N^{α}-benzyloxycarbonyl-N^{ϵ}-Boc-L-lysine from the *Z*-derivative by standard methods but including a recrystallization of the (—)-ephedrine salt,[354] spontaneous N^{ϵ}-methylation and N^{α}-formylation of lysine in formaldehyde solutions and their inhibition by ascorbic acid,[355] N^{ω}-2-(2-trimethylsilylethyl)oxycarbonylation of αω-diamino acid copper(II) complexes,[356] and conversion of ω-guanidino- and -ureido-α-amino acids into α-keto acids without disturbing the side-chain function (*via* 2-trifluoromethyloxazolin-5-ones).[357]

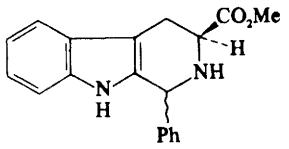
[348] S. E. Wu, W. P. Huskey, R. T. Borchardt, and R. L. Schowen, *Biochemistry*, 1983, **22**, 2828.
[349] M. J. Davies, B. C. Gilbert, and R. O. C. Norman, *J. Chem. Soc., Perkin Trans. 2*, 1983, 731.
[350] K. O. Hiller and K. D. Asmus, *J. Phys. Chem.*, 1983, **87**, 3682.
[351] A. Hanaki and H. Kamide, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2065.
[352] M. Chen, *Huaxue Shiji*, 1983, **5**, 173 (*Chem. Abstr.*, 1983, **99**, 140 338).
[353] A. A. Isab, *Inorg. Chim. Acta*, 1983, **80**, L3.
[354] S. T. Chen and K. T. Wang, *Org. Prep. Proced. Int.*, 1983, **15**, 361.
[355] L. Trezl, I. Rusznak, E. Tyihak, T. Szarvas, and B. Szende, *Biochem. J.*, 1983, **214**, 289.
[356] A. Rosowsky and J. E. Wright, *J. Org. Chem.*, 1983, **48**, 1539.
[357] C. Klein, G. Schulz, and W. Steglich, *Liebigs Ann. Chem.*, 1983, 1623.

α-Amino acids undergo *N*-nitrosation in either crystalline or solution forms when exposed to 1–100 p.p.m. nitrogen oxide-containing atmospheres.[358]

Aromatic side chains undergo modification in non-enzymic oxidation of 5-(*S*-cysteinyl)Dopa into a 1,4-benzothiazine under physiological conditions, mediated by complex formation with various metal ions (however, Cu^{2+}-catalysed oxidation leads to the red-purple pigment trichochrome F),[359] in *O*-alkylation[360] and *O*-phosphorylation of tyrosine under standard conditions,[361] and in electrochemical oxidation of *N*-acetyl tyrosinamide.[362] The tyrosine side chain is, however, the signal for the chymotrypsin-catalysed formation of *N*-acetyl-L-tyrosine ethyl ester from the *N*-acetylamino acid, ethanol, and the immobilized enzyme in a two-phase ($CHCl_3$–H_2O) system.[363]

Tricarbonylchromium complexes form a bridge between aromatic and heteroaromatic examples as far as the organization of this section of the chapter is concerned. Phenylalanine derivatives react with $Cr(CO)_6$ in refluxing water–THF (4:1) to give good yields of the complexes,[364] and tryptophan–$Cr(CO)_3$ complexes suitable for use in peptide synthesis are prepared similarly (but in Bu_2O–THF).[365] Tryptophan derivatives protected at amino and carboxy groups yield cyclic tautomers readily when a methoxycarbonyl *N*-protecting group is involved;[366] in contrast to earlier reports, however, *N*-benzylidene analogues do not readily cyclize to Pictet–Spengler products 1,2,3,4-tetrahydro-β-carbolines (17) in the absence of acids.[367] Free-radical halogenation of protected tryptophans using *N*-bromo- or -chloro-succinimides provides high yields of the 2-halo-substituted analogues.[368] Numerous mechanistic studies involving the histidine imidazole moiety as base catalyst in hydrolysis studies are discussed in the preceding section; the propensity of histidine itself (rather than larger structures containing histidine or imidazole groupings as discussed earlier[321–324]) to catalyse the hydrolysis of *p*-nitrophenyl acetate, though not a new topic for study, has been clarified through comparisons with the behaviour of *N*-methylhistidines.[369] Consideration is given in this study[369] to the protonation state and interactions between the amino and imidazole groups of the histidines, a complex matter

[358] C. Janzowski, R. Klein, R. Preussmann, and G. Eisenbrand, *Food Chem. Toxicol.*, 1982, **20**, 595.
[359] A. Palumbo, G. Nardi, M. D'Ischia, G. Misuraca, and G. Prota, *Gen. Pharmacol.*, 1983, **14**, 253.
[360] W. L. Mendelson, A. M. Tickner, and I. Lantos, *J. Org. Chem.*, 1983, **48**, 4127.
[361] P. F. Alewood, R. B. Johns, R. M. Valerio, and B. E. Kemp, *Synthesis*, 1983, 30.
[362] C. Jakubowicz, L. T. Yu, and J. A. Reynaud, *Electrochim. Acta*, 1983, **28**, 57.
[363] J. L. Vidaluc, M. Baboulene, V. Speziale, A. Lattes, and P. Monsan, *Tetrahedron*, 1983, **39**, 269.
[364] C. Sergheraert, J. C. Brunet, and A. Tartar, *J. Chem. Soc., Chem. Commun.*, 1982, 1417.
[365] C. Sergheraert and A. Tartar, *J. Organomet. Chem.*, 1982, **240**, 163.
[366] M. Taniguchi, A. Gonsho, M. Nakagawa, and T. Hino, *Chem. Pharm. Bull.*, 1983, **31**, 1856.
[367] R. H. Grigg, H. Q. N. Gunaratne, and E. McNaghten, *J. Chem. Soc., Perkin Trans. 1*, 1983, 185.
[368] R. S. Phillips and L. A. Cohen, *Tetrahedron Lett.*, 1983, **24**, 5555.
[369] P. Boschcov, W. Seidel, J. Muradian, M. Tominaga, A. C. M. Paiva, and L. Julio, *Bioorg. Chem.*, 1982, **11**, 383.



(17)

requiring assessment of equilibria of various kinds but crucial to underpin the reliability of conclusions drawn in this type of study.

Non-enzymic Models of Biochemical Processes Involving Amino Acids. – Amino acid–nucleotide complexes have been the mainstay of this section over the years and are mentioned elsewhere in this chapter. Reports of other related work describe dissociation by near-u.v. irradiation of DNA complexes with tyrosine, histidine, and tryptophan[370] and extension of stability-constant studies to mixed-ligand complexes $[M^{2+}(ATP)(aa)]^{3-}$ in assessment of metal-ion-promoted hydrophobic interactions between amino acids and nucleotides.[371]

Peptide synthesis has been achieved in aqueous media by reaction of glycine with *N*-acetylglycyl adenylate anhydride[372] and by reaction of tyrosine with the Schiff base of *N*-acetyltyrosinal and hydrogen peroxide.[373]

Effects of Electromagnetic Radiation on Amino Acids. – This section carries summaries of photochemical processes and of processes in which absorption of radiation does not lead to bond-breaking. In the former category, flash photolysis of pyridoxal-derived Schiff bases (yielding decarboxylation products from which pyridoxamine and carbonyl compounds are formed by hydrolysis[374]) and of aqueous tyrosine solutions (yielding degradation products of the initially formed *p*-alanylphenoxyl radical[375]) is accompanied by photo-oxidation studies of histidine using the traditional photosensitizer (Rose Bengal) covalently bonded to a polymer[376] and by photo-oxidation studies of the role of tyrosine and tryptophan as sensitizers in the formation of the superoxide radical anion $O_2^{-\bullet}$ in aqueous solutions.[377]

An unusual type of paper describes a study of radiation damage to crystalline L-valine during electron microscopy at liquid He temperature, which is only some 4–6-fold less than that at room temperature, rather than 70-fold as previously reported.[378] The more familiar studies of irradiation of solid amino acids are illustrated in γ-irradiated L-alanine single crystals which, as studied by e.s.r.

[370] E. M. Mil and V. M. Zhiltsova, *Izvest. Akad. Nauk S.S.S.R., Ser. Biol.*, 1983, 925.
[371] H. Sigel, B. E. Fischer, and E. Farkas, *Inorg. Chem.*, 1983, **22**, 925.
[372] D. W. Mullins and J. C. Lacey, *J. Mol. Evol.*, 1983, **19**, 173.
[373] B. L. Strehler, P. Schmid, M. P. Li, K. Martins, and H. Fliss, *J. Mol. Evol.*, 1982, **19**, 1.
[374] Y. Kurauchi, K. Ohga, S. Morita, T. Nagamura, and T. Matsuo, *Chem. Lett.*, 1983, 349.
[375] X. Shen, S. Pang, Y. He, and Y. Zhang, *Shengwu Huazue Yu Shengwu Wuli Xuebao*, 1983, **14**, 491 (*Chem. Abstr.*, 1983, **98**, 143 817).
[376] F. I. Llorca, J. L. Iborra, and J. A. Lozano, *Photobiochem. Photobiophys.*, 1983, **5**, 105.
[377] W. M. Draper and D. G. Crosby, *J. Agric. Food Chem.*, 1983, **31**, 734.
[378] M. K. Lamrik, D. A. Kopf, and J. D. Robertson, *Nature (London)*, 1983, **301**, 332.

spectroscopy, undergo deamination to form $MeC\dot{H}CO_2H$ radicals,[379] and there are similar studies[380] of γ-irradiated L-leucine. In this work the interest lies, as far as the authors are concerned, in the decay processes of these radicals; in the former case conformational reorientation seems to occur,[379] while a remarkable influence of the atmosphere surrounding the irradiated crystal has been demonstrated (decay in H_2 is much faster than decay in Ar) in the latter study.[380] E.n.d.o.r. evidence indicates the formation of radicals in irradiated *N*-acetylglycine originating at sites of crystal imperfections.[381]

Fluorescence lifetimes of various ionic forms of tryptophan in aqueous solutions are determined by H^+ quenching, and thus an explanation can be found for the longer lifetimes in ^2H_2O.[382] Addition of Et_3N or acid varies the corresponding fluorescence lifetimes observed for tryptophan in MeOH or EtOH.[382] A picosecond time-scale has been established for diffusion-controlled quenching of tryptophan fluorescence, and, since disturbance of the diffusion time by nearby ions and molecules can be thereby measured, parameters such as the interaction radius of the collisional quenching of *N*-acetyltryptophanamide by I^- can be determined.[383] 5-Methoxytryptophan yields a fluorescence spectrum that undergoes a pronounced red-shift as the pH of the solution is raised, owing to deprotonation of the $\dot{N}H_3$ group.[384] This is claimed to be the first direct evidence for the effect of a part of the aliphatic moiety on the energy of an emitting state of the indole moiety in tryptophan.

Tyrosine, and especially its 3,5-dihalo derivatives, is effective in quenching the phosphorescence of the triplet state of acetone.[385]

6 Analytical Methods

Gas-Liquid Chromatography. – A declining number of papers is appearing on this topic, and those that are published amount to extensions or improvements to established methodology. Commonly used perfluoroacyl and -alkyl *N*- and *O*-masking groups for conversion of amino acids into volatile derivatives have been compared,[386] it having been established[387] that *N*-trifluoroacetylamino acid n-propyl esters of 42 amino acids could be separated within about 19 min. Similar assessments of *N*-heptafluorobutyroyl amino acid propyl esters[388] and

[379] V. R. Zaitov, V. A. Onishchuk, and S. Z. Shul'ga in 'Proceedings of the Tihany Symposium on Radiation Chemistry', 1983, Vol. 5, p. 1037 (*Chem. Abstr.*, 1984, **100**, 22978).

[380] M. Mahdavi and M. Dole, *J. Phys. Chem.*, 1983, **87**, 5430; M. Dole and M. Mahdavi in 'Proceedings of the Tihany Symposium on Radiation Chemistry', 1983, Vol. 5, p. 1015 (*Chem. Abstr.*, 1984, **100**, 22977).

[381] F. Z. Khalaf and I. Miyagawa, *J. Chem. Phys.*, 1983, **78**, 5886.

[382] E. Gudgin, R. Lopez-Delgado, and W. R. Ware, *J. Phys. Chem.*, 1983, **87**, 1559.

[383] R. W. Wijnaendts van Resandt, *Chem. Phys. Lett.*, 1983, **95**, 205.

[384] E. F. Gudgin-Templeton and W. R. Ware, *Chem. Phys. Lett.*, 1983, **101**, 345.

[385] E. Rivas-Suarez, L. H. Catalini, E. J. H. Bechara, and G. Cilento, *Photochem. Photobiol.*, 1983, **37**, 93.

[386] G. Gamerith, *J. Chromatogr.*, 1983, **268**, 403.

[387] G. Gamerith, *J. Chromatogr.*, 1983, **256**, 267.

[388] S. A. Vitt, E. A. Paskonova, M. B. Saporovskaya, and V. M. Belikov, *Prikl. Biokhim. Microbiol.*, 1983, **19**, 692.

isobutyl esters[389] have been reported, with the analytical objectives of determining muramic acid, diaminopimelic acid, and alanine ratios in bacterial cell walls (employing electron-capture detection),[389] tryptophan at nanogram levels,[390] and 1-methylhistidine in physiological samples.[391]

Estimation of enantiomeric purity of amino acid samples by g.l.c. over chiral stationary phases is illustrated by well established Chirasil-Val g.l.c.,[392] corresponding use of chiral poly(siloxane)s (which have the merit of stability up to 230 °C),[393] and newer instrumental possibilities (two-column g.l.c.).[394]

Ion-exchange Chromatography. – The general area of liquid-chromatographic separation is now overlaid with the h.p.l.c. instrumental facility, and the traditional subdivisions of this section of the chapter are becoming less unambiguous. Readers are therefore directed also to the later h.p.l.c. section for continuation of this coverage of ion-exchange chromatography.

Conventional ninhydrin ion-exchange analysis[395–398] (including assay of pipecolic acid[396] and of tryptophan, 5-methyltryptophan, and glucosamine[397] and identification of EDTA as a source of enhanced ninhydrin colour intensities, probably due to decomposition products of the EDTA contaminant[398]) is being strongly challenged by *o*-phthaldialdehyde–mercaptoethanol reagent systems with fluorimetric quantitation (as illustrated in the estimation of β-hydroxyaspartic acid in protein hydrolysates;[399] see also the later h.p.l.c. section).

Automated amino acid analysis has been reviewed.[400]

Thin-layer Chromatography and Related Techniques. – Screening of physiological samples in a routine manner for specific species has been a major application of t.l.c.[401] and is illustrated[402] in estimation of tyrosine in serum employing fluorescence densitometry. Tyrosine, phenylalanine, and tryptophan derivatives have been subjected to t.l.c. in different solvent systems to determine optimum separation conditions.[403]

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The other papers are concerned with explorations of uncommon or new experimental approaches. Separation of amino acids on stannic tungstate[404] and on ammonium tungstophosphate[405] as ion-exchange materials shows some promise, the latter giving good separation of methyltryptophans. Partition systems ranging from the not unfamiliar polyamide materials (for the separation of *O*-phosphotyrosine, serine, and threonine[406]), reversed-phase materials (C_{18}-bonded silica gel, impregnated silica gel, and acetylated cellulose for the separation of amino acid mixtures, only the first-mentioned offering true reversal of the elution sequence but only when impregnated with dodecylbenzenesulphonic acid[407]), to glass-fibre paper (used in two-dimensional separations of ^{15}N-labelled amino acids[408]) have been tested. Adequate results are given within a short time using relatively small t.l.c. plates in amino acid analysis,[409] a feature emphasized for a pressurized ultra-micro chamber for miniaturized t.l.c., the effect here also being the reduced diffusion of spots.[410]

A mixed hydroxide of iron(III) and bismuth(III) is capable of preferential removal of acidic amino acids from aqueous solutions of pH 4.[411] A chelation principle is probably involved, and desorption can be achieved with Na_3PO_4 buffer at pH 12.2. The technique, used for the separation of tyrosine from aspartic acid, might be suitably adapted for t.l.c. analysis.

High-performance Liquid Chromatography. – Several reviews of this rapidly expanding area have appeared, concentrating solely on amino acid analysis,[412–416] although including some covering narrow aspects (practical procedures,[414] analysis of keto acids formed from amino acids through the use of amino acid oxidase[416]).

The subject appears to have progressed to the point where a few efficient systems have survived from a broad range of possibilities on which the preceding volumes of this Report have drawn. Views differ on whether pre- or post-column derivatization represents the best regime for h.p.l.c. of amino acids, although the preference is usually determined by the stability or otherwise of

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the pigments of fluorophores created by derivatization. Post-column methods are strongly advocated[413] and widely adopted for the dominant *o*-phthaldialdehyde–mercaptoethanol reagent system[417–430] (among these papers there are several[420–423] illustrating pre-column derivatization). There are many points of interest in these studies, but primarily that *o*-phthaldialdehyde fluorimetric amino acid analysis is being taken up widely in preference[425] to ninhydrin methodology. As many as 48 components were identified within 50 min in a standardized use of *o*-phthaldialdehyde for amino acid mixtures,[421] and a parallel study[422] establishes analysis routines for mixtures of 21 common amino acids (but proline, hydroxyproline, and cysteine cannot be detected). Imino acids, however, and other secondary amines, are specifically detectable through hypochlorite cleavage into primary amines in a post-column operation followed by *o*-phthaldialdehyde derivatization and fluorimetric assay.[426,427] Particular amino acids featured in these studies include glutamine,[429] γ-carboxyglutamic acid,[430] cysteinesulphinic acid and cysteic acid,[417] tyrosine and 3-fluorotyrosine,[418] and 1- and 3-methylhistidines.[419] Variations of standard technique include assessment of automated analysis[428] and reversed-phase ion-pair h.p.l.c.,[424] the latter approach also having been used in h.p.l.c. of dansylamino acids[431] (one of a number of papers based on these derivatives[431–434] and related[435] *N*-arenesulphonylamino acids). Post-column derivatization, while used in analysis of *S*-sulphocysteine in urine,[433] is an uncommon variation with these derivatives. Fewer papers have appeared dealing with h.p.l.c. analysis of phenylthiohydantoins;[436–438] they include an example of the unusual pre-column derivatization

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procedure using phenyl isothiocyanate for quantitative analysis of amino acid mixtures.[438] Further studies (Vol. 14, p. 31) of the use of 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole to generate fluorescent derivatives ($\lambda_{emission}$ 524–541 nm, $\lambda_{excitation}$ 467–472 nm) with amino acids (except tryptophan)[439] and of modification of the procedure appropriate for imino acid analysis[440] have been reported.

Surprisingly little interest, relatively speaking, is being shown in estimation of enantiomer purity by h.p.l.c. methods employing mobile phases containing copper(II)-L-amino acids. Separation in this form of resolution applied to dansylamino acids is based on equilibria between ternary complexes.[441,442]

Electrochemical detection[423,443,444] has been favoured for tyrosine[443] and 5-hydroxytryptophan[444] when biological samples were analysed for these amino acids. Related h.p.l.c. studies of aromatic amino acids deal with tryptophan[445–447] and its indoleamine metabolites in brain tissue[446] {sub-picomole amounts of tryptophan are best analysed through the fluorescence of its Pictet–Spengler reaction product, 9-hydroxymethyl-β-carboline, formed with HCHO and $K_3[Fe(CN)_6]$[447]}, Dopa[448] and 5-(*S*-cysteinyl)Dopa,[449] and phenylalanine.[450] Less common amino acids studied include *meso*-alanopine and strombine,[451] members of the mycosporine group,[452] mimosine,[453] and pyroglutamic acid (nanomole level assay as its 4-nitrophenacyl ester[454]).

Fluorescence Methods. – This section offers a safety net for methods not located in the preceding sections. The *o*-phthaldialdehyde–thiol system has been established to react with an amino acid to yield an isoindole. Triphenylmethanethiol has been studied for the first time in this context,[455] confirming the nature of the fluorescent adduct and establishing the effects of amino acid concentration on time to reach maximum fluorescence. The stability of this fluorophore varies with the structure of the amino acid, tryptophan, Dopa, and other amino acids

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that present a hindered environment to the $-NH_2$ group yielding longer-lived fluorescence.[456]

The fluorescent derivative formed between tryptophan and a modified Koshland reagent, 2-carboxy-1-hydroxy-4-naphthylmethyl dimethylsulphonium chloride, shows $\lambda_{emission}$ 416 nm after excitation at 255 nm. A useful aspect is that in acid solutions only tryptophan and cysteine react with this reagent but in neutral solutions histidine, methionine, and amino acids with hydroxy groups in their side chains also react.[457]

Serine levels in blood samples may be estimated by reaction with periodic acid and fluorimetric estimation of the resulting HCHO.[458]

The sole mention of fluoresceamine in this year's chapter, formerly achieving considerable popularity, refers to the estimation of lysine after its enzymic decarboxylation to cadaverine.[459]

Other Methods. – Chemiluminescence generated through reactions of the H_2O_2 formed in the amino acid oxidase-catalysed degradation of an amino acid is directly proportional to the concentration of the amino acid. Concentrations down to 2.5×10^{-7} M[460] or to more than 10-fold lower levels[461] are measurable, and the choice of the enzyme of appropriate specificity permits L-amino acids alone, or D-enantiomers, to be determined.

Potentiometric titration of Boc-amino acids mixed with amino acids gives curves that can be interpreted to reveal relative amounts of the two structural types.[462]

A specific interaction between some amino acids and Trisacryl GF05 gel can be exploited in analytical separations.[463]

Determination of Specific Amino Acids. – The L-amino acids implied can be selectively analysed using lysine decarboxylase immobilized on a CO_2-sensing electrode,[464] lysine oxidase[465] or tyrosinase[466] immobilized on a pO sensor, and asparagine using *Serratia marcescens* immobilized on an NH_3-sensing electrode.[467] Use of an enzyme not linked with an electrochemical assay is represented in L-tyrosine decarboxylase degradation of L-tyrosine and fluorimetry of the tyramine produced in this way.[468] An enzymic deamination method resulting in the conversion of 1-aminocyclopropane-1-carboxylic acid into α-ketoglutaric acid has been described.[469]

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2

Structural Investigations of Peptides and Proteins

BY R. CASSELLS, A. J. GARMAN, W. D. MERCER, R. H. PAIN, M. D. SCAWEN, R. A. G. SMITH, AND OTHERS

PART IA: Protein Isolation and Characterization

By M. D. Scawen, A. Atkinson, A. Electricwala, P. M. Hammond, and R. F. Sherwood

1 Introduction

There has again been a decrease in the number of publications describing the purification of proteins. Applications of high-performance liquid chromatography are frequently reported, but they are mainly concerned with the behaviour of synthetic mixtures of proteins on various matrices.

The format of this report is similar to that of previous years, although necessarily shorter. The majority of the data are presented in tabular format with the emphasis being on those proteins purified by affinity techniques.

2 Protein Isolation Methodology

Affinity Chromatography. – Table 1 lists proteins purified during 1983 in which affinity chromatography was employed at some stage.

The biospecific elution of enzymes from ion exchangers, using a substrate, has been employed for the purification of fructose bisphosphate aldolases from spinach and maize, which could be eluted from phosphocellulose by 1 mM fructose 1,6-bisphosphate at pH 7.3,[1] and a histamine *N*-methyltransferase from rat liver, which could be eluted from DEAE-Sephacel using 1 mM histamine.[2]

Coupling Techniques. Cyanogen bromide is still the most often cited activation method, but attempts to find a less toxic alternative continue to be made. One such compound, *p*-nitrophenylcyanate, has been proposed. This yields cyanate esters of agarose in the same manner as CNBr, but with a faster reaction rate. In addition the degree of substitution was readily monitored by determination of the *p*-nitrophenolate ion.[3]

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Table 1 *Proteins purified by affinity chromatography*

Protein	*Source*	*Matrix*	*Ligand*	*Eluent/Comment*	*Ref.*
Transglutaminase	Guinea-pig liver	CH-Sepharose	Phenylalanine	Bind in presence of 2 mM $CaCl_2$. Elute using 10 mM EDTA	8
Ribonuclease	Bovine semen	Sepharose	Adipolydihydrazo-ATP	1.0 M NaCl	9
Glucose 6-phosphate dehydrogenase	Human erythrocytes	Sepharose	$NADP^+$	0.1 mM $NADP^+$	10
NADPH–cytochrome C reductase	Rat liver	Agarose	ADP	0.15% deoxycholate	11
Tyrosine hydroxylase	Rat adrenal gland	Sepharose	Heparin	0.4 M KCl	12
Glutathione reductase	Gerbil liver	Sepharose	ADP	0.05–0.5 mM NADPH gradient	13
Choline acetyltransferase	Bovine brain	Sepharose	*N*-(10-Carboxy)decamethylene-4-(1-naphthylvinyl)-pyridine	0.2 M NaCl, 1 mM EDTA 2% EtOH, 25 mM sodium citrate, pH 5.7	14
Alcohol dehydrogenase	Rat liver	Sepharose	AMP	0.15 M NaCl, 5 mM NAD^+	15
Bile-salt-activated lipase	Human milk	Sepharose	(i) Cholate	2% cholate	16
		Sepharose	(ii) Heparin	0.3 M NaCl	
Ribonucleotide reductase	Human Detroit 98 cell line	Agarose	ATP	Enzyme elutes in void volume	17
Phospholipase A2	Bovine-ileum smooth muscle	Agarose	1-(12′-Aminododecanoyl)-2-hexadecyl-*sn*-glycero-3-phosphocholine	Enzyme bound in 10 M $CaCl_2$. Elute with 20 mM EDTA	18
Calcium-dependent protein kinase	Pig spleen	Agarose	Phosphatidyl serine	Bind in presence of 4 mM Ca^+. Elute with 2 mM EDTA, 4 mM EGTA, 2 M NaCl	19
Laccase (polyphenol oxidase)	Sycamore (*Acer pseudoplatanus*) cells	Sepharose	Concanavalin A	1% α-methyl mannopyranoside	20
Cystatin	Hen egg-white	Sepharose	*S*-Carboxymethylpapain	Bind at pH 6.5. Elute at pH 11.5, 0.5 M NaCl, 10% (v/v) glycerol	21

Cholinesterase	Horse or human serum	Sepharose	Procainamide	0–100 mM procain amine gradient	22
Cyclic nucleotide phosphodiesterase	Rat pancreas	Sepharose	Calmodulin	Binds in 0.2 mM $CaCl_2$. Elute with 0.1 mM EGTA	23
Phosphofructokinase	Rat intestine	Agarose	ATP	1 mM fructose 6-phosphate, 1 mM ADP	24
Renin	Human-kidney cortex	AH-Sepharose	Isosteric peptide inhibitor (D-His-Pro-Phe-His-Leu-Leu-Leu-Val-Tyr)	pH gradient, pH 6.0 to pH 3.2	25
Thy-1-glycoprotein	Mouse thymocytes	Sepharose	Concanavalin A	0.5 M methyl α-D-mannoside, 1 M ethylene glycol	26
Endoglucanase	*Aspergillus fumigatus*	Sepharose	Concanavalin A	20 mM α-methylmannoside	27

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Table 1 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluent/Comment*	*Ref.*
Alcohol dehydrogenase	Rat retina	CH-Sepharose	AMP	10 mM Tris HCl, pH 7.8	28
Aldehyde dehydrogenase	Bovine lens	Sepharose	NAD^+	1 mM NAD^+	29
Ribonuclease	Human seminal plasma	Agarose	4-(Aminophenyl)phosphouridine-2′-(3′)-phosphate	4 M NaCl	30
NADH dehydrogenase	*Bacillus* YN-1	Sepharose	5′-AMP	(i) 0.6 M KCl (ii) 0.3 mM NAD	31
Acetyl-CoA : arylamine *N*-acetyltransferase	Pigeon liver	AH-Sepharose	Amethopterin	0–1.5 mM amethopterin gradient	32
Dipeptidyl peptidase	Rat kidney	Sepharose	Concanavalin A	0–0.8 M gradient of methyl mannoside	33
NADPH : nitrate reductase	*Neurospora crassa*	Sepharose	FAD	1 mM FAD, 0.37 M NaCl	34
Aldehyde reductase	Rat kidney	Sepharose	$NADP^+$	0.5 mM $NADP^+$	35
Ornithine decarboxylase	Rat liver	AH-Sepharose	Pyridoxamine phosphate	0.2 mM pyridoxal phosphate	36
Thymidine kinases	BHK-21 cells infected with *Herpes simplex* virus	Sepharose	Thymidine	0.1–0.3 mM thymidine gradient	37
Glycogen synthase kinase	Rabbit liver	Sepharose	Calmodulin	1.5 mM EDTA	38
Chymotrypsin-like proteinase	Human skin	CH-Sepharose	D-Tryptophan methyl ester	25 mM D-tryptophan methyl ester	39
Adenylate cyclase	*E. coli*	Sepharose	ADP	0.02–0.5 M phosphate gradient + 5 mM ATP	40
Esterase A	Rat urine	Agarose	Aprotinin	0.1 M acetate buffer, pH 3.5	41
Calcium-dependent protease	Rat heart	Sepharose	Casein	400 mM KCl, 1 mM EGTA	42
Aldehyde dehydrogenase	Human liver	Sepharose	AMP	100 mM phosphate buffer, pH 7.0	43
Thymidine kinase	Human placenta	Sepharose	Thymidine	0.3 mM thymidine	44
Calcium-activated protease	Human platelets	AH-Sepharose	Antipain	0–0.35 M NaCl gradient	45

Ubiquitin-activating enzyme	Human erythrocytes	CH-Sepharose	Ubiquitin	1 M KCl followed by 2 mM AMP	46
Calcium-activated neutral proteases	Calf brain	CH-Sepharose	Casein	Bind in presence of 30 mM $CaCl_2$. Elute with 10 mM EGTA	47
N-Acetylmuranoyl hydrolase	*Streptococcus faecium*	Sepharose	Haemoglobin column in series with concanavalin A	50 mM α-methyl D-pyranoside	48
Choline acetyl transferase	Bovine brain	CH-Sepharose	*N*-Methyl-4-(1-naphthyl-vinyl)pyridine	0.2 M NaCl	49
Enkephalin convertase	Bovine pituitary glands	Sepharose	*p*-Aminobenzoyl-L-arginine	1 M L-arginine	50

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Table 1 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluent/Comment*	*Ref.*
Trypsin inhibitor	*E. coli*	Sepharose	Trypsin	0.1–100 mM HCl gradient	51
Folypolyglutamate synthetase	*L. casei*	Agarose	AMP	250 mM KCl	52
Pertussin toxin	*Bordetella pertussis*	Sepharose	Fetuin	4 M $MgCl_2$	53
Collagenase	Human leucocytes	Sepharose	Collagen	1.0 M NaCl	54
Lysozyme	Bacteriophage T4	Affigel 202	C6-muropeptide	1.0 M NaCl	55
5′-Nucleotidase	Bovine liver	Sepharose	5′-AMP	10 mM AMP	56
Papain	*Papaya* latex	Sepharose	Aminocaproyl-L-Leu-D-Phe	0.5 mM $HgCl_2$	57

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Table 2 *Proteins purified by dye-affinity chromatography*

Protein	*Source*	*Matrix*	*Ligand*	*Eluent/Comment*	*Ref.*
Nitrate reductase	*Chlorella*	Sepharose	Cibacron Blue F3G-A	Pretreat column with bovine albumin. Elute bound enzyme with 0.1 mM NADH	58
Dihydropterin reductase	Rat adrenal glands	Sepharose	Cibacron Blue F3G-A	0.1 mM NADH	59
Uridine phosphorylase	*E. coli*	Agarose	Procion Green HE-4BD (Matrex Gel Green A)	2 mM uridine	60
Alcohol dehydrogenase	Rat liver	DEAE-agarose	Cibacron Blue F3G-A (DEAE-Affi-Gel Blue)	10 mM NaCl, 10 mM Tris HCl, pH 6.5	15
Alcohol dehydrogenase	Rat liver	Agarose	Cibacron Blue F3G-A	0.15 M NaCl, 2.5 mM NAD^+, 10 mM Tris HCl, pH 6.5	15
Glycerokinase	*Bacillus stearothermophilis*	Sepharose	Procion Blue MX-3G	5 mM ATP	61
Glucose 6-phosphate dehydrogenase	*Leuconostoc mesenteroides*	Agarose	(i) Matrex Gel Purple A (ii) Matrex Gel Orange B	Enzyme does not bind 0.05 mM NADP	62
N-Acetyltyrosine deacetylase	Monkey kidney	Sepharose	Cibacron Blue F3G-A	0.5 M NaCl	63
Phosphofructokinase	*Aspergillus niger*	Sepharose	Blue Dextran	0–1 M $(NH_4)_2SO_4$ gradient	64
Citrate synthase	*Bacillus megaterium*	Agarose	Matrex Gel Red A	(i) 0–0.5 M NaCl gradient (ii) 0.1 mM CoA, 0.1 mM oxaloacetate	65

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Table 2 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluent/Comment*	*Ref.*
Hydrogenase	*Alcaligenes eutrophus*	Agarose	Procion Red HE-3B	0.1–2.0 M KCl gradient	66
Melvalonate kinase	Pig liver	Sepharose	Cibacron Blue F3G-A	0.2–1.5 M KCl gradient	67
NAD(P)H-cytochrome *c* reductase	*Chlamydomonas reinhardii*	Agarose	Cibacron Blue F3G-A	0.1 M KCl	68
Malate dehydrogenase	Pea	Sepharose	Procion Red HE-3B	0–3 M NaCl gradient	69
Glutamine synthetase	*Saccharomyces cerevisiae*	Agarose	Cibacron Blue F3G-A	0–250 mM potassium nicotinate gradient	70
Isovaleryl CoA dehydrogenase		Agarose	Cibacron Blue F3G-A	0–8 mM FAD gradient	71
Uridylyl transferase uridyl-removing enzyme	*E. coli*	Agarose	Cibacron Blue F3G-A	0.75–2.5 M KCl gradient	72
Putidaredoxin reductase	*Pseudomonas putida*	Sepharose	Cibacron Blue F3G-A	10 mM NADH	73
Aldose reductase	Rabbit muscle	Sepharose	Cibacron Blue F3G-A	0–1 M NaCl gradient	74
Acyl coenzyme A reductase	*Photobacterium phosphoreum*	Sepharose	Cibacron Blue F3G-A	0.2 M Hepes buffer, pH 7.5	75
Thymidine kinase	Human placenta	Sepharose	Cibacron Blue F3G-A	5 mM ATP	44
Benzene dihydrodiol dehydrogenase	Mouse liver	Agarose	Procion Red HE-3B	1.5 M NaCl	76
2-Methyl branched-chain acyl CoA dehydrogenase	Rat liver	Agarose	Cibacron Blue F3G-A	0.35 M NaCl + 5 mM FAD	77
N-Acetylglutamate synthetase	Rat liver	Agarose	Cibacron Blue F3G-A	0.5 M KCl	78
Pertussis toxin	*Bordetella pertussis*	Agarose	Cibacron Blue F3G-A	0.75 M $MgCl_2$	53
Lactate dehydrogenase	*Alcaligenes eutrophus*	Agarose	Matrex Green A Cibacron Blue F3G-A	0.25 M KCl, 0.5 mM NADH 75–750 mM KCl gradient	79
Aldehyde reductase	Chicken kidney	Sepharose	Cibacron Blue F3G-A	0.2 M NaCl	80
ATP–AMP phospho-transferase	*Paracoccus denitrificans*	Agarose	Cibacron Blue F3G-A	3 mM ATP, 3 mM EDTA	81

Alcohol dehydrogenase isoenzymes	Mouse liver	Sepharose	Procion Red HE-3B (A_2 isoenzyme)	0.5 mM NAD	82
			Procion Scarlet H-3G (B_2 isoenzyme)	0.5 mM NAD	
Alcohol dehydrogenase isoenzymes	Mouse stomach		Procion Blue HE-G followed by Procion Yellow MX-3R	0–0.2 M NaCl gradient, 1 mM NAD	
Hydroxy-steroid dehydrogenases	*B. fragila* and other bacteria	Sepharose	Procion Red HE-3B	0.6 M or 1.0 M NaCl	83

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Dye-affinity Chromatography. Affinity chromatography on immobilized dyes continues to be an important method for the purification of a wide variety of proteins, as listed in Table 2.

The recovery of a variety of enzymes, including nitrate reductase, malic dehydrogenase, and pyruvate kinase, from Cibacron Blue Sepharose was shown to be increased if the column matrix was pretreated with bovine serum albumin. However, the effect of presaturation with albumin on the degrees of purification obtained was not investigated.[4] Cibacron Blue has been attached to glycerol-propyl porous glass, and the resulting conjugate was shown to be an affinity matrix for lactate dehydrogenase.[5]

Two reviews have been published, covering various aspects of the interaction of Cibacron Blue F3G-A and other dyes with protein.[6,7]

Hydrophobic-interaction and Covalent Chromatography. — *Hydrophobic-interaction Chromatography.* During the past year there has been a significant decrease in the number of investigations that have been employed on hydrophobic-interaction chromatography steps during enzyme purification. The use of phenyl-Sepharose, however, has shown an increase, the majority of studies using this matrix. New matrices continue to be introduced and the range of available supports continues to expand. One of the major additions to those available is the new range of matrices from Toyo Soda (Tokyo, Japan). Kato *et al.*[84] describe a new phenyl-based matrix in which phenyl groups have been introduced into their silica-based TSK-G500PW gel[85] *via* an ether linkage. It has been successfully used to separate a variety of proteins under h.p.l.c. conditions.

Burley and Sleigh[86] describe the hydrophobic-interaction chromatography of avian apoproteins in urea solutions. The study of apoproteins has been largely complicated by isolation problems as they are substantially insoluble in aqueous buffers. The use of urea maintains a soluble solution, allowing the various apoproteins to be separated.

A new method for the purification of antiglycosphingolipid antibody using a hydrophobic matrix has been described.[87] Octyl-Sepharose was used as a support to which equine anti-haematoside was bound *via* a hydrophobic link. The glycolipid–octyl-Sepharose matrix was then used as an immunoadsorbent for affinity column chromatography to purify avian anti-haematoside antibody. This use of a hydrophobic matrix for producing an affinity matrix may find wider use in protein purification, particularly as the octyl-Sepharose matrix is capable of bonding very strongly with many protein materials.

Table 3 lists examples from the major biochemical journals published in 1983.

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Covalent Chromatography. There have been few reports of the use of covalent chromatography for protein isolation in the literature of 1983, although a small number has been published.

Macartney and Tschesche[88] describe the use of activated thiol-Sepharose in the formulation of a purification protocol for latent and active human polymorphonuclear leukocyte collagenases. Standard procedures employing cysteine in the elution buffer were used. The same matrix was used by Ryder and Eaker[89] for the identification of thiol groups in human ceruloplasmin.

Immunoaffinity Chromatography. – Immunoaffinity chromatography is rapidly becoming a powerful tool for the purification of proteins. The literature survey for 1983 has shown that a wide variety of proteins and enzymes have been purified by means of this technique, and a few examples of these are listed in Table 4.

The use of monoclonal antibodies for the preparation of immunosorbents and their subsequent use in protein purification continue to receive extensive study. Monoclonal antibodies are directed against specific antigenic determinants in the protein, and as such the proteins of interest are isolated with high yield and purity following immunoaffinity chromatography. For example, kidney endopeptidase[106] was purified in a single step by immunoaffinity chromatography with a yield of 40% and with specific activity comparable to that purified by other means. Similarly, surface protein of *T. gondii*,[107] rat-liver cytochrome 450,[108] human urokinase,[109] and porcine colipase[110] have all been successfully purified in a one-step immunoadsorption procedure using matrix-bound monoclonal antibodies.

The solid supports used in the preparation of immunosorbent have also been examined. Recently studies have shown that chemically modified supports seem to be superior to CNBr-activated Sepharose, in terms of both binding capacity and the yield of the purified protein. A novel method was described for the coating of potential column supports with alginate ester film. In a model experiment, sheep anti-human IgG was coupled to CNBr-activated film on Sepharose and the resultant immunosorbent was used to purify IgG from whole human serum.[111] In another study, agarose polyaldehyde microsphere beads were produced by encapsulating polyaldehyde microspheres with agarose. The beads containing the reactive aldehyde groups were used for covalent coupling of proteins or antibodies. The conjugate was then used successfully for the preparation of immunosorbent.[112]

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Table 3 *Proteins purified by hydrophobic-interaction chromatography*

Protein	*Source*	*Ligand/Matrix*	*Chromatography conditions*		*Ref.*
			Loading conditions	*Elution conditions*	
Elastin	Ovine lung and vascular tissue	Alkyl-agarose	20 mM NaP, pH 7.4, 0.2 M NaCl	(i) 50% DMF (ii) 1% SDS	90
Epoxide hydrolase	Mouse liver	Phenyl-Sepharose	100 mM Tris, pH 7.6, 0.35 M NaCl	Reducing NaCl gradient	91
5-Enolpyruvyl shikimate 3-phosphate synthase	*E. coli*	Phenyl-Sepharose	100 mM Tris, pH 7.5, 0.8 M $(NH_4)_2SO_4$	Reducing $(NH_4)_2SO_4$ gradient	92
Metalloendopeptidase	Rat brain	Phenyl-Sepharose	50 mM Tris, pH 8.0, 20% saturation, $(NH_4)_2SO_4$	Reducing $(NH_4)_2SO_4$ gradient	93
Diphtheria toxin	*Corynebacterium diphtheriae*	Phenyl-Sepharose	Culture supernatant containing 13% (w/v) $(NH_4)_2SO_4$	10 mM NaCl in 10 mM phosphate, pH 7.5	94
Uroporphyrinogen decarboxylase	Human erythrocytes	Phenyl-Sepharose	2 mM KP, pH 7.0, 2 M $(NH_4)_2SO_4$	Ethanediol in 2 mM KP, pH 7.0	95
20 000-Da light chain of myosin	Poultry smooth muscle	Phenyl-Sepharose	20 mM MOPS, pH 7.5, 0.6 M NaCl	H_2O	96
Carnitine acetyltransferase	*Candida tropicalis*	Octyl-Sepharose	50 mM KP, pH 7.5, 45% saturation, $(NH_4)_2SO_4$	Stepwise reduction of $(NH_4)_2SO_4$ to zero	97
Enolase isoenzymes	Yeast	Phenyl-Sepharose	10 mM Tris, pH 9.0, 10% ethanediol, 45% saturation, $(NH_4)_2SO_4$	Increasing gradient of ethanediol (10–50%), reducing gradient of $(NH_4)_2SO_4$ (45–0%)	98
Uterine progesterone receptor	Rabbit	Aminoalkyl-agarose	0.6 M NaCl TESH Mo	Decreasing NaCl gradient (0.6 M to 6 mM) or increasing ethanediol gradient (0–40%)	99
Colony-stimulating factor	Human urine	Phenyl-Sepharose	4 M NaCl in PBS, pH 7.2	Stepwise reduction of NaCl	100

Lipoprotein	Human plasma	Phenyl-Sepharose	50 mM Tris/saline, pH 7.6	Stepwise increase of propylene-glycol	101
Neutral protease	Rabbit skeletal muscle	Phenyl-Sepharose	20 mM Tris, pH 7.5, 0.3 M NaCl	Reducing gradient of NaCl, increasing gradient of ethanediol	102
Myeloperoxidase eosinophil peroxidase	Human erythrocytes	Phenyl-Sepharose	Sodium acetate, pH 5.6, 0.5 M $CaCl_2$, 0.05% CTAB, 25% glycerol	Loading buffer containing 70% glycerol	103
Aryl acylamidase	*Pseudomonas fluorescens*	Phenyl-Sepharose	0.3 M KP, pH 7.6	Wash with Tris HCl and elute with decreasing Tris gradient	104
Naphthalene dioxygenase	*Pseudomonas* sp.	Octyl-Sepharose	Tris HCl, pH 7.8, 10% ethanol, 10% glycerol, 40% $(NH_4)_2SO_4$	Loading buffer containing 30% $(NH_4)_2SO_4$	105

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Table 4 *Proteins purified by immunoaffinity chromatography*

Protein	*Source*	*Eluent/Comments*	*Ref.*
Procolipase A	Porcine	Elution with 0.53 M formic acid	114
β-Endorphine precursors	Porcine	Use of β-lipotropin directed monoclonal antibodies to detect and purify β-endorphine precursors	115
Prostacycline synthase	Bovine	Elution with 0.1 M MES, pH 6, containing 0.5% Triton X-100, 1 M NaCl, and 2 mM 2-mercaptoethanol	116
Colipase	Human	Two forms of enzymes were isolated	110
Lipoxygenase-1 and -2	Soybean	Pulse elution with 2 M or 3 M ammonium thiocyanate or 0.1 M glycine HCl buffer, pH 2.5	117
Urokinase	Human	Use of monoclonal antibodies. Elution with 0.2 M acetate buffer, pH 3, containing 0.15 M NaCl and 0.1% Tween 80	109
Cytochrome P450	Rat	Separation of isozymes by use of monoclonal antibodies. Elution with 0.1 M glycine, pH 3	108
Interleukine 1	Murine macrophage cell line	IgG to IL1 did not inhibit IL2. Large-scale immunosorbent purification of IL1	118
Membrane protein (P30)	*Toxoplasma gondii*	Use of monoclonal antibodies to surface immunodeterminants and to purify antigen. Elution with 0.1 M diethylamine, pH 11.5, and 0.5% sodium cholate	107
IgG	Human	Sepharose alginate–ester support for immunosorbent	111
Albumin	Bovine	Novel immunosorbent based on agarose polyaldehyde microsphere beads. Elution with 0.2 M glycine HCl, pH 2.4	112
Endopeptidase	Pig	Monoclonal antibodies for immunosorbent preparation	106

The conditions required for the specific elution of proteins from immunosorbent vary considerably with different proteins. A novel procedure was described that involved the desorption of antigen with distilled water combined with an interruption period during the desorption procedure.[113]

Phase Partition. – An aqueous two-phase system has been described for the rapid purification of human fibroblast interferon. Using the phosphate ester of

[113] D. Bureau and J. Daussant, *J. Immunol. Methods*, 1983, **57**, 205.
[114] J. Rathelot, P. Delori, and L. Sourda, *Biochim. Biophys. Acta*, 1983, **742**, 39.
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[116] D. L. DeWitt and W. L. Smith, *J. Biol. Chem.*, 1983, **258**, 3285.
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polyethylene glycol in a phosphate/NaCl mixture resulted in a 350-fold purification.[119]

High-performance Liquid Chromatography. – Two major works on h.p.l.c. have been published in the current year.[120,121] The first, a review book, contains articles on the h.p.l.c. of peptides and proteins and the selection of mobile phases.[120] The second, published as one issue of *J. Chromatogr.*,[121] presents the major papers of the Third International Symposium on the h.p.l.c. of proteins, peptides, and polynucleotides held in November 1983 in Monte Carlo. This volume is rich in data covering all aspects of protein and peptide separation, analysis, and preparative-scale purification. It is neither the place nor the intention of this review to cover data as readily obtainable in a single issue. However, the volume covers the purification of many proteins by a combination of ion exchange, gel permeation, and reversed-phase and pseudo-affinity (triazine dye) chromatography on a wide selection of different matrices.

In other literature a potentially exciting advance in matrix development, synthesis of a new polymeric bonded matrix of polyanamide covalently bonded to silica, to make a readily derivatizable matrix, has been described.[122] The readily prepared, pH-stable, cation-exchange matrix was shown to purify bacitracin, chymotrypsinogen, cytochrome C from various sources, and lysozyme. Subsequently the analysis of human-blood Hb types using a bistris gradient was demonstrated on this novel matrix.[123] Another development in h.p.l.c. technique has been the demonstration of linearity of elution position (time) with molecular weight on gel permeation on tandem I-250 and I-125 columns over the molecular weight range 0.5×10^6 to 2×10^4 at *low* ionic strength at approximately neutral pH.[124] Even fibrous proteins with axial ratios of 50 eluted according to their molecular weight and independent of their Stoke's radius. Another development described in the same paper[124] was the use of a single mixed I-250/I-125 shorter and wider column with excellent resolution under the same conditions. This type of column system may lead the way to truly preparative h.p.l.c. gel permeation. In reversed-phase chromatography two anomalies have been reported, firstly that the Ca^{2+} binding proteins, the parvalbumins, do not elute at positions consistent with their hydrophobicity[125] and secondly that ternary solvents containing acetonitrile plus propanol caused later elution of rhodopsin and cytochrome P450 than either alone or on phenyl and C_{18} matrices.[126]

In research applications, the purification of metallothioneins on gel-permeation, reversed-phase (C_8: propanol in Tris, pH 7.2), and ion-exchange (DEAE: gradient, 10–250 mM Tris, pH 7.2) h.p.l.c. matrices under conditions that

[119] U. Menge, M. Morr, U. Mayr, and M. R. Kula, *J. Appl. Biochem.*, 1983, **5**, 75.
[120] 'High Performance Liquid Chromatography: Advances and Perspectives', ed. C. Horvath, Academic Press, 1983, Vol. 3.
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[122] S. Gupta, E. Pfannkoch, and F. E. Regnier, *Anal. Biochem.*, 1983, **128**, 196.
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[124] J. A. Barden, *Anal. Biochem.*, 1983, **135**, 52.
[125] M. W. Berchtold, C. W. Heizmann, and K. J. Wilson, *Anal. Biochem.*, 1983, **129**, 120.
[126] G. E. Tarr and J. W. Crabb, *Anal. Biochem.*, 1983, **131**, 99.

prevent dissociation of the bound metal has been described.[127] The very rapid (2 minute) separation of α-acid glycoprotein, cytochrome C, ovalbumin, and μ-lactoglobulin on wide-pore polyethyleneimine silica employing an increasing phosphate and decreasing pH gradient has been demonstrated,[128] whilst an acetonitrile–methanol–phosphate system has been shown to separate the zeta and various gamma chains of Hb Portland I and to yield quantities of up to 5 mg of the globin chains.[129]

Other potentially significant h.p.l.c. separations reported include the separation of the histone proteins from hamster cells,[130] the preparative isolation of distinct molecular forms of ferritin,[131] and the separation of kallikrein from urine.[132] The molecular-weight determination of peptides in the molecular-weight range 10^3–10^4 in 6 M guanidine has also been described.[133]

Two publications from the same authors dealing with mechanism and multi-column systems[134,135] have covered the fact that for good separation the selection of the mobile phase, particularly in ion exchange, is probably more crucial than with conventional soft-gel systems[134] and that separation systems can be designed and affected in a multi-column separation system employing the full breadth of h.p.l.c. matrices of gel permeation, reversed phase, and anion and cation exchange.[135] The use and value of the system were demonstrated with synthetic protein mixtures.

Other Chromatographic Techniques and Applications. – Magnetic media offer a number of advantages as affinity adsorbents. A novel polyaldehyde microsphere, encapsulated with agarose in the presence of ferrofluid, gives such an adsorbent, which has been used for the rapid isolation of antibodies and antigens.[112]

Non-specific interactions are a frequent complication in affinity-chromatography techniques, and an investigation into the source of such interaction has been carried out. The matrix, the covalently coupled protein, and the loaded protein were examined as possible sources of non-specific interference.[136]

α_2-Macroglobulin is an effective inhibitor of proteinases. By taking advantage of the affinity of α_2-macroglobulin for Zn^{2+} a 'sandwich' affinity technique was developed from crude extracts. α_2-Macroglobulin was immobilized onto Zn-chelate Sepharose and the resulting complex used for the batch-wise removal of proteinases.[137]

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3 Isolation of Specific Classes of Proteins

Membrane Proteins. – The isolation and study of membrane proteins have continued to play an important role in protein chemistry, although the number of notable publications has reduced a little in 1983. A new series of books on membrane proteins, consisting of three volumes to date, has been published. The series is devoted to the structure and biochemical basis of receptor action and outlines the procedures involved in such studies. It is the first notable series on receptor studies for almost a decade. The first volume[138] deals with membranes and detergent solubilization of receptor proteins, the second volume[139] outlines purification methodology, and the third[140] describes characterization studies. An international meeting on the 'Structure and Function of Membrane Proteins' was held,[141] which may be of interest to many membrane-protein chemists.

Most membrane proteins are solubilized in the presence of a detergent compound, and it is often necessary to determine the molecular weight of such proteins in the presence of the surfactant. Maezawa *et al.*[142] describe a technique for the determination of the molecular weight of membrane proteins by using low-angle laser light scattering and h.p.l.c., in the presence of non-ionic surfactants.

The types of detergent used to solubilize membrane proteins have not changed; Triton X-100 remains the most used agent. Other agents continue to be investigated, often as part of studies on the improved solubilization of a specific protein.[143,144]

The continued use of neutral, non-denaturing aryl polyoxyethylene ethers still presents the problem of detergent removal, often necessary for subsequent purification studies. An organic solvent extraction may be employed to remove detergent. Salcedo *et al.*[145] describe the use of isopentanol to remove detergents from hydrophobic bacterial proteins, a technique that may find wider application in the study of membrane proteins. A further method frequently employed is the use of Bio-Beads SM-2, for example, to remove Triton. A simple, rapid technique is described by Horigome and Sugano[146] for removal of Triton and cholate from multiple samples.

As in previous years, animal tissue provides the major source of membrane proteins investigated, although there has been a significant increase in the

[138] 'Receptor Biochemistry and Methodology 1: Membranes, Detergents and Receptor Stabilisation', ed. J. C. Venter and L. C. Harrison, Alan R. Liss Inc., 1984.
[139] 'Receptor Biochemistry and Methodology 2: Receptor Purification Procedures', ed. J. C. Venter and L. C. Harrison, Alan R. Liss Inc., 1984.
[140] 'Receptor Biochemistry and Methodology 3: Molecular and Chemical Characterisation of Membrane Receptors', ed. J. C. Venter and L. C. Harrison, Alan R. Liss Inc., 1984.
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Table 5 *Purification of membrane proteins*

Protein	*Source*	*Solubilizing agent*	*Purification studies*	*Ref.*
Enkephalinase A and B	Calf brain	Triton X-100	DEAE-cellulose	147
Laminin	Human placenta	Triton X-100	Gel filtration	148
Actin polymerization stimulator	Bovine thyroid	Triton X-100	DNase I affinity chromatography	149
Δ^4-Steroid-5α-reductase	Rat epidermal tissue	Lubrol WX	Sucrose density sedimentation	150
Serine protease	Walker 256 carcinoma cells	Triton X-100	Electrophoretic and h.p.l.c studies	151
Thy-1 glycoprotein	Chicken brain	Na-deoxycholate	Lentil lectin Sepharose, Sephacryl S-200 SDS-p.a.g.e.	152
Bacteriophage T4 particle-associated lysozyme	Bacteriophage T4e mutant	Triton X-100	(i) Rivanol precipitation Amberlite CG 501 (ii) Muropeptide C6-agarose (iii) CM-Sephadex C-25	153
Alkylglycerol mono-oxygenase	Rat liver	Triton X-100	Aminohexyl-Sepharose, DEAE-cellulose, sucrose density centrifugation	154
α-Limit dextrinase	Porcine intestine	Freeze–thaw cycles	DEAE-cellulose, $(NH_4)_2SO_4$ fractionation, Sepharose 4B Bio-Gel HTP, DEAE-Sepharose CL-6B	155
Enkephalinase	Porcine brain	Triton X-100	Monoclonal antibody GK 7C2 immunoadsorbent chromatography	156
Hydrogenase	*Alcaligenes eutrophus* H16	Not stated	$(NH_4)_2SO_4$ fractionation, Red HE-3B agarose, DEAE-Sephacel	157
Protein 4.1	Human erythrocytes	Tween 20	DE-52 cellulose, Sephadex G-150	158
Glycoproteins IIb and IIIa	Human platelets	Triton X-114	DEAE-cellulose, SDS treatment, h.p.l.c. on TSK-4000 SW	159
L-CAM adhesion protein	Chicken liver	Trypsin	DEAE-cellulose, Sephadex G-150, isoelectricfocusing	160
Succinate dehydrogenase	Rat liver	Triton X-100	Study of effect of detergents on enzyme release	161
Myelin	Bovine brain	Triton X-100	Study of effect of detergents on protein release	162
Citrate carrier protein	Rat liver, bovine heart, rat brain	Triton X-100	Hydroxyapatite	163

Phosphate carrier protein	Porcine heart	Triton X-114	Hydroxyapatite	164
Dopamine receptor	Porcine pituitary gland	Digitonin	Preliminary studies on lectin agarose and Sephadex G-50	165
Calmodulin	AH-66 hepatoma	Lubrol PX	Sephadex G-100, fluphenazine-Sepharose, 4B affinity chromatography, SDS-p.a.g.e.	166
Disulphide isomerase	Rat liver	Triton X-100	Heat treatment, CM-Sephadex, DEAE-Sephacel	167

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Table 5 *(cont.)*

Protein	*Source*	*Solubilizing agent*	*Purification studies*	*Ref.*
Surface protein P30	*Toxoplasma gondii*	Nonidet P-40	Electrophoretic studies and immunoabsorption studies with monoclonal antibodies	168
Sialoglycoprotein SGP 140	P-12 Ichikawa lymphoblastoid cells	Triton X-100	DEAE-Sephacel, WGA-agarose, Blue Sepharose, Sephadex G-150	169
ATP synthetase	Chloroplast thylakoid membrane	Triton X-100	Preliminary electrophoretic studies	170
Prolactin receptor	Rat liver	CHAPS	Gel filtration on Sepharose 6B	171
ATPase	Rabbit-muscle sarcoplasmic reticulum	Dodecyl octaethylene glycol monoether	Detergent solubilization studies, prior to kinetic investigations	144
Enterotoxin (*Clostridium perfringens*) binding protein	Rabbit intestine	Nonidet P-40	Enterotoxin-Sepharose affinity matrix	172
Hydrogenase	*Rhodopseudomonas capsulata*	Triton X-100	PEG precipitation, DE-52 cellulose, SDS-p.a.g.e.	173 174
Collagen-binding glycoprotein	Chick chondrocyte membrane	Trypsin	Collagen-Sepharose affinity matrix	175
Phorbol diester receptor	Rat brain	Divalent-ion chelation	$(NH_4)_2SO_4$ fractionation, DEAE-cellulose, AcA 44 agarose	176
Enkephalin aminopeptidase	Rat brain	Triton X-100	DEAE-Sepharose, Bio-Gel HTP, Sephadex G-200	177
Cell envelope proteins	*Haemophilus influenzae*	Triton X-100	pH precipitation, SDS-p.a.g.e.	178
RNA polymerase	Pea chloroplasts	Triton X-100	Discontinuous glycerol gradient fractionation, DEAE-cellulose, Sephadex 6B	179
Cytochrome C	*Rhodopseudomonas capsulata*	Triton X-100	Sephadex G-50, DEAE chromatography, full details not given	180
Laminin	Murine BL6 melanoma	Triton X-100	Affinity chromatography and preliminary electrophoretic studies	181

Hexokinase	Rat brain	Glucose 6-phosphate	Preliminary electrophoretic studies prior to kinetic investigations	182
Arylsulphatase	Human placenta	Triton X-100	Hydroxyapatite, chromatofocusing, DEAE-Sepharose	183
Cyclic AMP phosphodiesterase	*Dictyostelium discoideum*	Butanol extraction and Triton X-100	Concanavalin A-Sepharose, DE-52 cellulose, isoelectric focusing, gel filtration	184
Insulin receptors	Murine 3T3-L1 adipocytes	Triton X-100	Affinity crosslinking with ^{125}I-insulin-disuccinimidyl suberate prior to SDS-p.a.g.e.	185
Hydrogenase	*Desulphovibrio desulphuricans*	Na-DOC	$(NH_4)_2SO_4$/pancreatin, first DEAE-Sephacel, second DEAE-Sephacel, hydroxyapatite	186
Phosphatidylinositol synthase	*Saccharomyces cerevisiae*	Triton X-100	CDP diacylglycerol-Sepharose, chromatofocusing	187

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Table 5 *(cont.)*

Protein	*Source*	*Solubilizing agent*	*Purification studies*	*Ref.*
Acetyl CoA-α-glucosaminide *N*-acetyl transferase	Human liver	Triton X-100	Concanavalin-A, Sepharose	188
Zeanalenone reductase	Rat liver	Triton X-100	DEAE-Sephadex, hydroxyapatite, Sepharose 4B	189
α-(1-3)-D-Mannosyl-transferase	Bovine pancreas	Triton X-100	T.l.c. studies, Bio-Gel P-6, h.p.l.c. studies	190
Placental mono-oxygenase	Human placenta	Triton N-101	Polyethylene glycol fractionation	191
Adenosine-binding sites	Rat brain stem	Na-CHO Na-DOC	Study of various detergents for optimum extraction conditions	192
Glial fibrillary acidic protein	Bovine brain	Triton X-100, urea	CM-Sepharose CL-6B, DE-52 cellulose, DNA-cellulose	193
Farnesyl pyrophosphate synthetase	Porcine liver	Triton X-100	Thin-layer radiochromatography, Sephadex G-200	194
Receptor sialoglyco-protein for Sendai virus	Bovine erythrocyte	Lithium di-iodosalicylate	Phenol partition, ethanol precipitation, phosphocellulose chromatography, SDS-Sepharose CL-4B	195
DNA-binding protein	*Bacillus subtilis*	Triton X-100	Hydroxyapatite, DEAE-cellulose, Sephacryl S-200	196
Na^+/D-glucose cotransporter protein	Rabbit intestine	Deoxycholate digitonin	Monoclonal-antibody affinity chromatography, $(NH_4)_2SO_4$ fractionation	197

Mitochondrial tricarboxylate carrier	Rat liver	Triton X-100	Hydroxyapatite	198
β-Lactamase	*Bacillis cereus*	Triton X-100	DEAE-Sephadex	199
Calmodulin-binding protein	Bovine brain	Lubrol PX	DEAE-Sephacel, calmodulin-Sepharose	200

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Table 6 *Purification of plasma proteins**

Protein	*Source*	*Major procedures*				*Ref.*
		Affinity chromatography	*Ion exchange*	*Gel filtration*	*Others*	
Proteins involved in coagulation and fibrinolysis						
Factor VIIIC	Human		Polyanionic/hydrophobic matrix			201
Thrombin enhancement factor	Human	Wheatgerm agglutin, Affi-Gel Blue	DEAE-cellulose DE-52	Sephacryl S-300		202
Antithrombin III	Guinea pig	Heparin-Sepharose	DEAE-Sephadex	Sephadex G-100		203
Antithrombin III	Equine	Affi-Gel Blue	DEAE-cellulose DE-52	Sephacryl S-300		204
Protein C and protein S	Human	Anti-Factor X, imm. chrom.	DEAE-Sepharose		Prep. p.a.g.e.	205
Prekallikrein	Porcine	*p*-Aminobenzamidine aminocaproic-Sepharose 4B	CM-Sephadex C-50			206
LMW kininogen	Human	Affi-Gel Blue	Sephadex QAE A-50	Sephadex G-200	Phenyl-Sepharose	207
LMW kininogen	Rat		DEAE-Sephadex A-50, CM-cellulose	Sephadex G-200	$(NH_4)_2SO_4$ ppt.	208
LMW kininogen	Human	Imm. chrom.	DEAE-Sephadex A-50	Ultrogel ACA-44		209
Complement and associated proteins						
C4	Human				Immunoprecipitation	210
C5	Human	Imm. chrom. ω-amino-hexyl-agarose			Hydroxyapatite	211
C1 inhibitor	Human	Lysine-Sepharose	DEAE-Sephadex A-50, CM-Sephadex C-50	Sephadex G-150	Hydroxyapatite	212
Factor B	Human		DEAE-Sephadex CL-6B, QAE-Sephadex A-50			213
Factor D	Human		CM-Sephadex C-50	Sephadex G-75		213

Lipoproteins

Apolipoprotein B	Human		Density-gradient ultracent., SDS-p.a.g.e.	214
Apolipoproteins	Human		Chromatofocusing	215
Apolipoproteins I and II	Human	H.p.l.c. on G3000SW TSK gel		216
Apolipoprotein A-IV	Human		Absorption on phospholipid triglyceride emulsion	217
HDL2 and HDL3	Human		Ultracentrifugation	218
'A' esterase	Sheep		Prep. p.a.g.e.	219

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Table 6 *(cont.)*

		Major procedures				
Protein	*Source*	*Affinity chromatography*	*Ion exchange*	*Gel filtration*	*Others*	*Ref.*
Apoprotein C	Human				Ultra-thin-layer isoelectricfocusing	220
Enzymes and inhibitors						
Amine oxidase	Pig	Con A-agarose	DEAE-cellulose	Biogel A1.5 M	Hydroxyapatite	221
Alkaline phosphatase	Human				Isoelectricfocusing	222
Enkephalin-degrading enzyme	Human	Con A-Sepharose Sepharose-Blue Dextran		Sephadex G-75		223
Cholinesterases	Horse Human	Procainamide Affinity gel	DEAE-Sephadex		Prep. p.a.g.e.	224
Acyltransferase	Human		DEAE-cellulose		Citric acid ppt., $(NH_4)_2SO_4$ ppt., hydroxyapatite	225
α2-Macroglobulin	Horse	Affi-Gel Blue	TEAE-cellulose	Sephadex G-200, Sephacryl S-300		226
Cysteine proteinase inhibitor	Bovine	Papain-Sepharose 4B	DEAE-Sephadex A-50	Sephadex G-150	$(NH_4)_2SO_4$ ppt.	227
Factor inhibiting G1-S transition	Rat, human		DEAE-cellulose DE-32		H.p.l.c. high-vol. electrophoresis	228
Growth-inhibiting factor	Mouse	Con A-agarose	DEAE-Biogel A	Sephacryl S-300		229
PCA-blocking factor	Rat	Imm. chrom.	DEAE-cellulose, CM-cellulose			230

Other plasma proteins

Sex-steroid-binding globulin	Human	Cortisol-Sepharose, Con-Sepharose		Biogel P300	$(NH_4)_2SO_4$ ppt.	231
Thyroxine-binding prealbumin	Human	Affi-Gel Blue	DEAE-Sephadex	Ultrogel ACA-44		232
Thyroxine-binding globulin	Human	Imm. chrom.	DEAE-Sephadex			233
Mannan-binding protein	Human	Mannan-Sepharose 4B		Sepharose CL-6B		234
Aldosterone-binding protein	Human			Sephadex G-200		235
$\alpha 2$-$\beta 1$-Glycoprotein	Horse	Sepharose-haemoglobin, Sepharose-Con A	DEAE-cellulose	Sephadex G-100/ Sephacryl S-300		236

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[236] A. Pellegrini, H. R. Zweifel, and R. von Fellenberg, *Int. J. Biochem.*, 1983, **15**, 1013.

Table 6 *(cont.)*

Protein	*Source*	*Major procedures*				*Ref.*
		Affinity chromatography	*Ion exchange*	*Gel filtration*	*Others*	
Neutral microprotein	Human	Imm. chrom.		Sephacryl S-200		237
C reactive protein	Human		DEAE-cellulose DE-52			238
IgE	Horse	Imm. chrom.		Sephacryl S-200		239
IgM	Sheep	Affi-Gel Blue	DEAE-Sephadex A-50	Sephacryl S-300		240
Ceruloplasmin	Sheep		DEAE-cellulose DE-52, QAE-Sephadex A-50	Biogel P-100	$(NH_4)_2SO_4$ ppt.	241
Fibronectin	Human	Gelatin-Sepharose, fibrin-Sepharose	DEAE-cellulose	Sepharose 6B-CL		242

* Abbreviations used in Table 6: prep. p.a.g.e. preparative polyacrylamide gel electrophoresis, h.p.l.c. high-pressure liquid chromatography

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number of reports of membrane proteins isolated from bacterial sources. Table 5 lists the investigations of membrane proteins reported in the major biochemical journals during 1983.

Plasma Proteins. – A wide range of proteins have been isolated and purified from the plasma of various mammalian species. The number of proteins isolated from plasma continues to grow with the application of novel and more sensitive techniques. This section highlights some of the active areas of plasma-protein research. Table 6 lists some of the proteins isolated during 1983 together with their major purification procedures. For clarity the listing has been broadly divided into five subsections.

The literature survey has shown that increasingly, with many proteins, the traditional protein-isolation methods are either replaced by or used in conjunction with an affinity-chromatography step, such as metal chelate, immunoaffinity, lectin-coupled agarose, *etc.* The applications of most of these kinds of chromatography are reviewed in this chapter. It seems that with most proteins the purified material can be isolated by a method involving fewer steps with a concomitant increase in yield and purity.

4 Electrophoretic Techniques

The major electrophoretic techniques and the detection and extraction of proteins from gel systems are reviewed, with references, up to early 1983 in a recent issue of *Methods Enzymol.*[243] Advances and applications in this field over the last year are described in the following subsections.

One-dimensional Electrophoresis. – One recent application of electrophoresis of proteins through polyacrylamide gels has been towards a more direct study of protein structure. Protein conformation and folding can be studied using slab gels in which conditions are varied in a gradient perpendicular to the direction of electrophoresis. Urea (0–8M) and temperature gradients have been used to study in this manner five bovine proteins, including ribonuclease A and α-lactalbumin.[244] A gradient of 2-mercaptoethanol set up between adjacent slab-gel tracks of protein with and without the reducing agent was used to identify inter- and intra-molecular disulphide bonds and was particularly successful in an analysis of immunoglobulin heavy and light chains.[245] Preferential cleavage of aspartyl-prolyl peptide bonds upon heating of protein samples at 110 °C has been used with SDS-p.a.g.e. to detect such bonds in peptide-mapping studies of fructose 1,6-bisphosphatases from a variety of mammals.[246] Analysis of small peptide fragments has been achieved by concentrating samples by buffer discontinuity and separating in 40–50% polyacrylamide gels.[247] An acidic (pH 3.0)

[243] W. B. Jakoby, *Methods Enzymol.*, 1984, **104**, 237.
[244] D. P. Goldenberg and T. E. Creighton, *Anal. Biochem.*, 1984, **138**, 1.
[245] R. J. Allore and B. H. Barker, *Anal. Biochem.*, 1984, **137**, 523.
[246] J. Rittenhouse and F. Marcus, *Anal. Biochem.*, 1984, **138**, 442.
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gel and an alkaline (pH 9.0) gel are described with resolution down to dipeptides. Improvements in separation of small proteins and polypeptides (2500–90 000) using discontinuous SDS-p.a.g.e. are described in a system run at pH 7.8 with the separating gel containing 8M urea at acrylamide concentrations from 5 to 18%.[248] Calibration curves were linear regardless of acrylamide concentrations, and the technique should prove valuable in the more difficult separation range below 15 000 daltons.

Two-dimensional Electrophoresis. – Application of high-resolution two-dimensional polyacrylamide gels to the study of complex polypeptide mixtures has continued with examples as diverse as nuclear proteins of human meningioma cells[249] and amniotic-fluid polypeptides.[250] Use of tetramethylurea and dimethylethyleneurea to solubilize more fully membrane proteins prior to electrophoresis has been made in the study of myelin proteins.[251] An evaluation of the isoelectric-focusing conditions of two-dimensional gels, where five parameters were systematically varied, highlighted the inconsistencies that can occur when gel-to-gel comparisons are made, and it recommended that optimized patterns were obtained at increased concentrations of NaOH (50mM) and H_3PO_4 (25mM) and durations in excess of 10 000 V-hours were run.[252] On the hardware side, developments in image enhancement and improved image processing by microcomputer, particularly using internal standards for reference, seek to minimize gel-reading variations.[253–255]

Isoelectric Focusing. – A procedure using 2.5% acrylamide–0.5% agarose gel has been demonstrated as being applicable to high-molecular-weight proteins using ferritin–IgG and ferritin–soybean agglutinin conjugates in excess of M_r 600 000 as standards.[256] I.e.f. has continued to find applications for the study of human proteins such as plasminogen,[257] α_2-macroglobulin,[258] and high-density lipoprotein.[259] Its use may be extended to run under dissociating conditions by the introduction of urea and neutral detergents,[260] provided the effect on pK values of the various reactive monomers in the system is taken into account to allow calculation of corrected pH gradients.

Affinity and Immunoelectrophoresis. – Preparative affinity electrophoresis was applied to the isolation of human-erythrocyte carbonic anhydrase using an

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[253] W. Schneider and J. Klose, *Electrophoresis*, 1983, **4**, 284.
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[256] R. M. Pino and T. K. Hart, *Anal. Biochem.*, 1984, **139**, 77.
[257] D. Dykes, M. Nelson, and H. Polesky, *Electrophoresis*, 1983, **4**, 417.
[258] S. A. Back and J. A. Alhadeff, *J. Chromatogr.*, 1983, **278**, 43.
[259] C. Luley and H. Watanabe, *J. Chromatogr.*, 1983, **278**, 412.
[260] E. Gianazza, G. Artoni, and P. G. Righetti, *Electrophoresis*, 1983, **4**, 321.

inhibitor, *p*-aminomethylbenzenesulphonamide, coupled to a carboxyl-agarose matrix.[261] Three human-plasma cholinesterase phenotypes were studied in polyacrylamide gels containing a derivative of *m*-amino-(substituted)-phenyltrimethylammonium salts by comparing apparent dissociation constants from the mobilities of the variants-*versus*-ligand concentration.[262]

Two variations of crossed immunoelectrophoresis have been described.[263, 264] In the former, analysis of insoluble membrane proteins is performed in the presence of 2% sodium dodecyl sulphate. Use of polyethylene glycol in the antibody-containing gel to enhance precipitin formation enables quantitative determination down to 5 ng protein. In the latter, a single process combines crossed-line immunoelectrophoresis with crossed-line immunoelectrofocusing to determine simultaneously isoelectric points while identifying individual antigens in a complex extract from *Candida tropicalis*.

Isotachophoresis. – Concentration of dilute samples of protein using isotachophoresis prior to high-performance liquid chromatography, where injection volumes are small, is an attractive development,[265] and an on-line system where concentrated protein can be pumped directly to gel-filtration columns has been described.[266]

Protein Determination in Electrophoretic Gels. – Sensitivity of silver staining has been improved about ten-fold by staining gels with Coomassie Blue and then washing with glutaraldehyde prior to silver staining,[267] or by blue toning with a mixture containing ferric chloride, potassium hexacyanoferrate(III), and oxalic acid following silver staining,[268] or by overstaining and then using an improved destaining procedure to visualize <0.01 ng protein mm^{-2},[269] or by repeated exposure of gel to hypo- and hyper-tonic solution to remove SDS and to improve influx of stain.[270]

Use of horseradish peroxidase for enzymatic detection of proteins on gels and nitrocellulose filters has been widespread. It has been used alone for visualizing glycoprotein–concanavalin A complexes in gels *via* binding of its own sugar moiety to the second active binding site on ConA[271] and conjugated to protein A for the detection of human α-fetoprotein.[272] Its use following transfer of protein to nitrocellulose filters has been assessed when coupled to second antibody,[273] and the sensitivity has been amplified by the use of avidin–biotin

[261] N. Bergenheim, U. Carlsson, and C. Hansson, *Anal. Biochem.*, 1983, **134**, 259.
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immunoperoxidase conjugates as demonstrated by detection of angiotensin-I-converting enzyme[274] and *Toxoplasma* membrane antigens.[275]

Recovery of proteins from gels on a milligram scale has been achieved using displacement electrophoresis,[276] and a simple electrophoretic extraction system operating under acid, neutral, or alkaline conditions has been described.[277] A quantitative method for measuring protein in solution by reaction with trinitrobenzene sulphate was compared with the Lowry and Bradford methods, and it claimed increased sensitivity and smaller variation through relative independence of amino-acid composition.[278]

PART IB: Chemical Modification of Proteins

By R. Cassels, A. J. Garman, and R. A. G. Smith

1 Introduction

The published literature on the chemical modification of proteins has continued to grow steadily, and, as in previous reports, we have attempted to summarize significant developments in the Table. A large number of routine modifications with established reagents have been omitted.

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* Abbreviations used in the following Table: CMC 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)-carbodi-imide metho-*p*-toluenesulphonate, DCCI *N,N'*-dicyclohexylcarbodi-imide, DEP diethylpyrocarbonate, DFP di-isopropyl phosphorofluoridate, DNS-Cl 5-dimethylaminonaphthalene 1-sulphonyl chloride, DTNB 5,5'-dithiobis-(2-nitrobenzoic acid), EDC 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide, EEDQ *N*-ethoxycarbonyl, 2-ethoxy 1,2-dihydro quinoline, FDNB 1-fluoro-2,4-dinitrobenzene, FITC fluorescein isothiocyanate, IAEDANS *N*-iodoacetyl-*N'*-(1-naphthyl-5-sulphonic acid) ethylene diamine, NBS *N*-bromosuccinimide, NEM *N*-ethylmaleimide, PCMB *p*-chloromercuribenzoic acid, PMSF phenylmethylsulphonyl fluoride, SPDP *N*-succinimidyl 3-(2-pyridyldithio)propionate, TNBS 2,4,6-trinitrobenzenesulphonic acid. Residues in parentheses indicate that the site of modification is uncertain.

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[274] J. J. Lanzillo, J. Stevens, J. Tumas, and B. L. Fauburg, *Electrophoresis*, 1983, **4**, 313.
[275] K. Ogata, M. Arakawa, T. Kashara, K. Shioiri-Nakano, and K. Hiraoka, *J. Immunol. Methods*, 1983, **65**, 75.
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[278] A. K. Hazra, S. P. Chock, and R. W. Albers, *Anal. Biochem.*, 1984, **137**, 437.

Table *Chemical modification of proteins**

Protein	*Source*	*Reagent*	*Residue*	*Comment*	*Ref.*
Acetylcholine receptor	PC-12 nerve cells	Dithiothreitol, DTNB, NEM, [4-(*N*-maleimido)benzyl]-α-trimethyl ammonium iodide, bromoacetylcholine	Cys_2	Essential disulphide, affinity labelling	1
Acetylcholine receptor	Locust supraoesophageal ganglion	4-(*N*-Maleimido)-[^{3}H]benzyl-trimethylammonium	(Cys)	Receptor-affinity labelling	2
Acetylcholine receptor	*Torpedo californica*	Succinimidyl 4-(*p*-maleimido-phenyl) butyrate	Cys, Lys	Close to 43 K subsynaptic protein	3
Acetylcholine receptor	*Torpedo californica*	[^{3}H]Quinacrine mustard		Binding site located	4
Acetylcholine receptor	*Torpedo mamorata* and *californica*	[^{3}H]Triphenylmethylphos-phonium ion, u.v.		Photoaffinity labelling	5
Acetylcholine receptor	Three *Torpedo* species	[^{3}H]Azidophencyclidine		Species differences in labelling pattern	6
Acetylcholine receptor, acetylcholinesterases	Various	*N*,*N*-Dimethyl-2-phenylaziridium ion		Inactivation kinetics	7
Acetylcholinesterase	*Torpedo californica*	*S*-Mercuric *N*-dansylcysteine	Cys	Four Cys modified without inactivation	8
Acid protease	*Rhizopus chinensis*	Various diazoketone spin labels	$-CO_2H$	Active site probed	9

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
ACTH peptides	Synthetic	3-Trifluoromethyl-3-(*m*-[^{125}I]-iodophenyl)diazirine		Hydrophobic photolabel	10
Actin	Rabbit	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole		Fluorescence probe for actin assembly	11
Actin	Rabbit	*N*-Pyrenyliodoacetamide		Monomer–polymer subunit exchange	12
Actin	Rabbit	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole	Lys	Effect of pH and polymerization	13
Actin	Rabbit	Dimethylsuberimidate	Lys	Interaction with DNase I	14
Actin, tropomyosin	Rabbit	Various fluorescent alkylating agents	Cys	Fluorescence-energy transfer	15
Actin	Rabbit	(i) Performic acid (ii) TNBS	(i) Cys, Met, Trp (ii) Lys	Effects on antibody induction	16
Actin	Rabbit	*N*,*N*′-*p*-Phenylene bismaleimide		Kinetics of dimer functions studied	17
F-Actin	Rabbit muscle	IAEDANS Fluorescein *S*-maleimide	Cys	Fluorescence study of polymerization	18
Actinidin	*Actinidia chinensis*	Benzofuroxan	Cys-25	*O*-Benzoquinone dioxime and Cys-sulphenic acid formed	19
Aconitase	Bovine heart	Various nitroxide spin-labelled sulphydryl reagents	Cys	NO to FeS distance 10–13 Å	20
Adenylate cyclase	Bovine brain	Azido[^{125}I]iodo-calmodulin		150K subunit binds	21
S-Adenosylhomocysteinase	Rat liver	DEP	His	Essential His	22
S-Adenosylhomocysteinase	Rat liver	Phenylglyoxal	Arg	Inactivation	23

ADP/ATP carrier protein	Bovine-heart mitochondrion	Various photoaffinity derivatives of atractyloside	Topography of binding sites	24
α_1-Adrenergic receptor	Rat-brain cortex	4-Amino-6,7-dimethoxy-2-{4-[*S*-(3-[^{125}I]iodo-4-azidophenyl)-pentanoyl]-1-piperazinyl} quinazoline	79K species labelled	25
α_1-Adrenergic receptor	Rat liver	2-[4-(4-Azidobenzoyl)piperazin-1-yl]-4-amino-6,7-dimethoxy quinazoline	Photoaffinity label	26
α_1-Adrenergic receptor	Rat liver	[^{3}H]Phenoxybenzamine	Selective labelling	27
β-Adrenergic receptor		*p*-Azido-*m*-[^{125}I]iodobenzyl-carazolol	Photoaffinity labelling	28

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
β-Adrenergic receptor	Rat, hamster	*p*-Azido-*m*-[^{125}I]iodobenzyl carazolol		Receptor heterogeneity due to proteolysis	29
β-Adrenergic receptor	Heart (various species)	*p*-Azido-*m*-[^{125}I]iodobenzyl-carazolol		Photoaffinity labelling; β_1 and β_2 receptors similar	30
β-Adrenergic receptor	Frog erythrocyte membrane	(±)-15-(4-Azido-3-[^{125}I]-iodobenzyl)carazolol		Photoaffinity probe	31
β-Adrenergic receptor	Lymphoma cells	[^{125}I]Iodoazidobenzylpindolol		Functional and structural heterogeneity demonstrated	32
Albumin	Bovine serum	[^{14}C]Acetaldehyde	Lys	Mechanism of ethanol toxicity	33
Albumin	Bovine serum	Melatonin/formaldehyde	$-NH_2$	C-2 and indole nitrogen alkylated	34
Albumin	Bovine serum	Periodate-oxidized *S. cerevisiae* cell walls	$-NH_2$	Hapten-carrier immunogen	35
Albumin	Bovine serum	*N*-Methyl-9-hydroxy-ellipticine/peroxidase	(Asp)	Biotransformation study	36
Albumin	Bovine serum	Formate and ethanol radicals	Cys_2	Aggregates formed by intermolecular —S—S— bonds	37
Albumin	Bovine serum	2-Carboxy-1-hydroxy-4-naphthylmethylsulphonium chloride	Trp	New fluorescent Koshland reagent	38
Albumin	Human serum	3,3′,4′,5-Tetrachloro[^{14}C]-salicylanilide		Labelling site identified	39
Albumin	Human serum	NBS	Trp	Trp drug-binding role	40
Albumin	Human and bovine serum	Haematoporphyrin, photoirradiation	Trp	Singlet oxygen intermediate	41

Alcohol dehydrogenase	Horse liver	D-Fructose, D-glucose	Lys-315	Enzyme efficiency increased	42
Alcohol dehydrogenase	Horse liver	3-Chloroacetylpyridine–adenine dinucleotide		Affinity labelling	43
Alcohol dehydrogenase	Horse liver	*S*-2-Chloro-3-(imidazol-5-yl) propionate	Cys-46	Enantioselective labelling	44
Alcohol dehydrogenase	Horse liver	Tetramethylcyclobutadiene nickel dichloride		Inactivation, 8 mol Ni mol^{-1}	45
Alcohol dehydrogenase	Horse liver	DEP	His	Inactivation	46
Alcohol dehydrogenase and others	Various	FMN-sensitized photodynamic modification		More efficient in multi-subunit proteins	47
Aldehyde dehydrogenase	Sheep liver	[^{14}C]Disulphiram	Cys	Drug acts as redox catalyst	48

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Aldolase	Rabbit skeletal muscle	*o*-Phthalaldehyde	Cys-336	Isoindole ring formation inactivates	49
Alkaline phosphatase	Calf intestine	Iodoacetamide	Cys	Concentration-dependent effects	50
Alkaline proteases	Various bacteria	Dansyl fluoride	Ser	Ser-Trp distance determination	51
D-Amino acid oxidase	Hog kidney	Cyclohexanedione	Arg	Active-site Arg	52
D-Amino acid oxidase		Nitroethane/cyanide		Suicide inhibition	53
D-Amino acid oxidase	Porcine kidney	DNS-Cl	His	Isolation of labile imidazolium adduct	54
4-Aminobutyrate aminotransferase	Porcine brain	6-Iodoacetamide fluorescein		Activity retained	55
Aminopeptidase	Porcine liver	Various thiol reagents	Cys	Kinetics of inactivation	56
Amine oxidase (Cu)	Bovine plasma, lentil	Phenylhydrazine		Cu, O_2 not needed for inactivation	57
β-Amylase	Soybean	DTNB, iodoacetamide	Cys	Interaction with cyclohexadextrin and maltose	58
Angiotensin-converting enzyme	Rabbit lung	HCHO, $NaB(CN)H_3$	Lys	Essential Lys; Cl^- protects	59
Angiotensin II/liver receptor	Rabbit	Disuccinimidyl suberate	$-NH_2$	Receptor identification	60
Angiotensin II receptor	Bovine adrenals	[Sar^1,(4′-N_3)Phe^8]angiotensin II		Photoaffinity label	61
Anion transporter	Bovine platelets	4,4′-Di-isothiocyanatostilbene 2,2′-disulphonate		Involvement in platelet aggregation	62
Anion transporter	Human erythrocyte	Phenylgloxal, cyclohexane-1,2-dione	Arg	Anions protect	63
Anion transporter	Human erythrocyte	Phenyl isothio[^{14}C]cyanate	Lys-32	N-Terminal 72 residues sequenced	64

β_1-Anticollagenase	Human plasma	Iodoacetamide	Cys	Essential Cys	65
Antithrombin III	Human plasma	Dimethyl-(2-hydroxy-5-nitrobenzyl)sulphonium bromide	Trp	Study of heparin–AT III interaction	66
Apoferritin	Sheep spleen	1,5-Difluoro-2,4-dinitrobenzene		Subunit dimer formation	67
Arginine aminopeptidase (Cl^-)	Rat liver, human erythrocyte	DTNB	Cys	Essential Cys	68
Aromatase	Human placenta	[^{3}H]Norethisterone		Thyroxine protects	69
Asparagine *N*-glycosyltransferase	Calf-liver microsomes	Arg-Asn-Gly-epoxyethylglycine-Ala-Val-OMe		Specific inhibition implies enzyme assistance	70

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Aspartate amino-transferase	Porcine heart	4-*N*-(2,4-Dinitro-5-fluorophenyl)-pyridoxamine 5′-phosphate	Lys	*Via* C-5 of 2,4-dinitro-phenyl	71
Aspartate amino-transferase	Porcine heart	*N*-(Bromoacetyl)pyridoxamine	Lys	Inactivation	72
Aspartate amino-transferase	Porcine heart	Potassium nitrodisulphonate	Trp-140	Spin-probe substrate analogue	73
Aspartate amino-transferase	Porcine-heart mitochondria	*N*-(Bromoacetyl)pyridoxamine (and phosphate ester)	Lys	Affinity labelling	74
Aspartic proteinase	*Fusarium moniliforme*	1,2-Epoxy-3-(*p*-nitrophenoxy)-propane Diazoacetyl-DL-norleucine *methyl* ester	($-CO_2H$)	Characterization	75
Aspartyl-tRNA synthetase	*S. cerevisiae*	Glucose, glucose 6-phosphate		Affinity labelling	76
ATPase	Bovine heart	DCCI	($-CO_2H$)	F_0 subunit site not influenced by F_1 subunit	77
ATPase	Bovine heart	3′-*O*-{3-*N*-[(4-Azido-2-nitro-phenyl)amino]propionyl} 8-azidoadenosine 5′-triphosphate		α–β subunits crosslinked	78
ATPase	*Neurospora crassa*	(i) DCCI (ii) 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole	(i) $-CO_2H$ (ii) Tyr	Different membrane sources compared	79
ATPase	Sarcoplasmic reticulum	*N*-(3-Pyrene)maleinimide	Cys	Fluorescence study of oligomer	80
ATPase	Rabbit sarcoplasmic reticulum	*N*-(1-Oxy-2,2,6,6-tetramethyl-4-piperidinyl) iodoacetamide		Spin-label study	81
ATPase (BF_1)	*E. coli*	4-Azido-2-nitrophenyl phosphate		P_i site on β-subunit labelled	82
ATPase (Ca^{2+})	Rabbit muscle	[^{3}H]Adamantane diazirine/$h\nu$		Hydrophobic photolabelling	83

ATPase (Ca^{2+})	Rabbit muscle	Dithiobis(succinimidyl propionate)	(Lys)	Oligomerization and inactivation distinguished	84
ATPase (Ca^{2+}, Mg^{2+})	Rabbit sarcoplasmic reticulum	*N*-(1-Anilinonaphthyl-4)maleimide, *N*-[*p*-(2-benzimidazoyl)phenyl]-maleimide, *N*-(7-dimethylamino-4-methyl-3-coumarinyl)-maleimide, 4-maleimide 2,2,6,6-tetramethylpiperidino-oxyl	Cys	Evidence for a Ca^{2+}-dependent conformational change	85, 86
ATPase, dATPase	*Drosophila melanogaster*	α-[^{32}P]dATP, α-[^{32}P]ATP, γ-[^{32}P]ATP		Direct photolabelling of 174K component	87
ATPase F_1	Bovine-heart mitochondria	8-Azido-[2-^{3}H]ATP		Labelling sites identified	88

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
ATPase F_1	Bovine	Quinacrine mustard		β-Subunit labelled: chloropromazine analogue	89
ATPase F_1	*E. coli*, bovine-heart mitochondrion	EEDQ, DCCI	$-CO_2H$	EEDQ inhibits Mg^{2+} binding	90
ATPase F_1	*Micrococcus lysodeikticus*	TNBS, methyl 12-O-(azido-formyl)stearate		No lipid contact in membrane	91
ATPase F_1	*Rhodospirillum rubrum*	8-Azido-ATP		Same binding site for hydrolysis and phosphorylation	92
ATPase F_1	Thermophilic bacterium PS3, *E. coli*	DCCI	Glu	Effects of ADP and Mg^{2+}	93
ATPase $F_B(H^+)$	Bovine-heart mitochondria	NEM	Cys	P_i–ATP exchange inhibited	94
ATPase $F_0(H^+)$	*E. coli*	Dithiobis(succinimidyl-proprionate)	$-NH_2$	Topology study	95
ATPase (H^+)	*E. coli*	EEDQ	$-CO_2H$	β-Subunit only modified	96
ATPase (H^+)	*Neurospora crassa*	Carbodi-imides		Only hydrophilic carbodi-imides inactive	97
ATPase (H^+)	*Neurospora crassa*	NEM		Inhibition, effect of Mg^{2+}	98
ATPase (H^+)	*Neurospora crassa*	[^{14}C]NEM	Cys	Essential SH	99
ATPase (H^+, K^+)	Porcine gastric mucosa	Allyl isothiocyanate	(Cys)	Possible inhibition of acid section	100
ATPase (H^+)	*Rhodospirillum rubrum*	Woodward's reagent K DCCI	$-CO_2H$	Two types of essential $-CO_2H$	101
ATPase (H^+-, K^+-transporting)	Porcine gastric microsomes	Carboxypyridine disulphide, *N*-(1-naphthyl)maleimide	$-SH$	ATP protects against inactivation	102
ATPase MF_1	Bovine heart	[^{14}C]-7-Nitro-2,1,3-benzoxadiazole, DCCI	Tyr-197	Tyr may be linked to Glu-199 by DCCI	103

ATPase (Na^+, K^+)	*Bufo marinus* kidney	*O*-Hexanoyl-3,5-di-iodo-*N*-(4-azido-2-nitrophenyl)tyramine		Hydrophobic photoaffinity label	104
ATPase (Na^+, K^+)	Dog kidney	3′-*O*-{3-[(4-Azido-2-nitrophenyl)-amino]-3-tritiopropionyl}ATP Cr^{III}		Photoaffinity labelling	105
ATPase (Na^+, K^+)	Dog kidney	ATP 2′,3′-dialdehyde		Affinity labelling	106
ATPase (Na^+, K^+)	Dog kidney	1,5-Difluoro-2,4-dinitrobenzene, 4,4′-difluoro-3,3′-dinitrophenyl sulphone, *o*-phenanthroline Cu^{2+}	Lys	Subunit associations studied	107
ATPase (Na^+, K^+)	Electric eel	*p*-Aminobenzenediazonium derivative of ouabain		Photoaffinity labelling	108

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
ATPase (Na^+, K^+)	Lamb kidney	2-(4′-Maleimidylanilino)naphthalene 6-sulphonic acid, *N*-(1-pyrene)maleimide	Cys	Pyrene derivative reacts with more hydrophobic SH	109
ATPase (Na^+, K^+)	Porcine kidney	[^{3}H]-24-Azidodigitoxoside		Photoaffinity labelling	110
ATPase (Na^+, K^+)	Porcine kidney	*N*-[*p*-(2-Benzimidazoyl)phenyl]-maleimide		Effect of phosphorylation on conformation	111
ATPases (Na^+, K^+), (Ca^{2+})		Various ATP analogues		Analysis of inactivation kinetics	112
ATPase (R_rF_1)	*Rhodospirillum rubrum*	DEP	His, Tyr	Inactivation correlates with His modification	113
ATP-binding protein	Rabbit neutrophil, rabbit skeletal muscle	8-Azido-[α-^{32}P]ATP		Photoaffinity labelling	114
ATP-binding sites	Rabbit-muscle sarcoplasmic reticulum	8-Azido-[α-^{32}P]ATP		53K, 105K, and 106K Dalton glycoproteins labelled	115
ATP synthases	Bovine-heart mitochondrion, *E. coli*	Photoreactive lipids		Membrane topology	116
ATP synthase	Spinach chloroplast thylakoid membrane	2-Azido-[β-^{32}P]ADP		Binding-site identification	117
ATP synthase F_0	*E. coli*	[^{125}I]Iodonaphthylazide	Cys-21	Unsuitable for membrane topology studies	118
ATP synthase (F_1F_0)	*E. coli* K12	1-Palmitoyl 2-(2-azido-4-nitro)-benzoyl *SN*-glycero-3[^{3}H]-phosphocholine		F_0 subunit b label located in hydrophobic region	119
ATP synthetase	Lettuce choloroplast thylakoid membrane	3′-*O*-(4-Benzoyl)benzoyl ADP/u.v.		α- and β-subunits modified	120
ATP synthetase F_0	*E. coli*	Various reagents		Domain structure	121
ATP translocase	*Torpedo marmorata*	Azido-[^{3}H]AMP, azido-[^{32}P]ATP		Photoaffinity labelling	122

Bacteriorhodopsin	*Halobacterium halobium*	4-Amino-2,2,6,6-tetramethyl-piperidine-*N*-oxyl, EEDQ	$-CO_2H$	Membrane-bound carboxyls located	123
Bacteriorhodopsin	*Halobacterium halobium*	7-Chloro-4-nitrobenz-2-oxa-1,3-diazole	Lys-41	Hydrophobic fluorophore	124
Bacteriorhodopsin	*Halobacterium halobium*	Dansyl hydrazine + carbodi-imide	$-CO_2H$	Flexibility of carboxyl tail	125
Bacteriorhodopsin	*Halobacterium halobium*	3-([1-^{14}C]Diazoacetoxy)-*trans*-retinal		Chromophore–protein crosslinking	126
Bacteriorhodopsin	*Halobacterium halobium*	EEDQ/3-nitrotyrosine methyl ester	$-CO_2H$	CO_2H near chromophore	127
Bacteriorhodopsin	*Halobacterium halobium*	Merocyanines	Lys-216	Chromophoric Schiff-base probes	128

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Bacteriorhodopsin	*Halobacterium halobium*	Tetranitromethane	Tyr	Light dependence examined	129
Band-3 protein	Erythrocyte	4,4′-Di-isothiocyanodihydrostilbene 2,2′-Sulphonate FDNB	Lys	Two Lys modified	130
Batroxobin	*Bothrops atrox*	2,4-Bis(*O*-methoxypolyethylene glycol)-6-chloro-*s*-triazine	Lys	Antigenicity decreased	131
b–c_1 complex	Beef-heart mitochondria	Tetranitromethane DEP DCCI	Tyr His $-CO_2H$	Effects on electron transport	132
Benzodiazepine receptors	Calf cerebral cortex	Benzodiazepine analogues		Specific photoaffinity labelling	133
Benzodiazepine receptors	Calf cerebral cortex	DEP	His	Diazepam but not GABA protects	134
Bile salt-binding polypeptides	Rat-intestine membranes	(7,7-Azo-3α,12α-dihydroxy-5β cholan-24-oic acid, 3β-azido-3α,12α-dihydroxy-5β-cholan-24-oic acid, (11ξ-azido-12-oxo-3α,7α-dihydroxy-5β-cholan-24-oyl)-2-aminoethane sulphonic acid		Rearrangeable carbene and nitrene precursors used	135
Bile acid transport system	Rat hepatocytes	7,7-Azo-3α,12α-dihydroxy-5β-cholan-24-oyl)-taurine, *N*-(4-azido-2-nitrophenyl)-taurine		Photoaffinity labelling in normal and transformed cells	136, 137
β-Bungarotoxin synaptic membrane protein	Snake venom/chick brain	*N*-Succinimidyl-6-(4′-azido-2′-nitrophenylamino) hexanoate		116K adduct	138
α-Bungarotoxin	*Bungarus multicinctus*	$HCHO/NaCNBH_3$	$-NH_2$	Difference labelling	139

Ca^{2+} channel	Guinea pig	2,6-Dimethyl-3,5-dicarbomethoxy-4-(2-isothiocyanatophenyl)-1,4-dihydropyridine		Inhibition, affinity labelling	140
Ca^{2+} pump	Human erythrocyte	FITC		Catalytic and regulatory ATP sites modified	141
Calmodulin	Bovine	Dansyl chloride		Probe of hydrophobic ligand binding	142
Calmodulin	Rabbit	5-({[(Iodoacetyl)amino]ethyl}-amino)-naphthalene-1-sulphonic acid		Immunoaffinity purification and characterization	143
Calmodulin	Ram testis	Various aromatic azides	Lys, Arg	Activation of several target proteins	144
Calmodulin/β-endorphin	Porcine brain	Bis-(sulphosuccinimidyl) suberate	Lys	Crosslinking	145

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Carbamyl phosphate synthetase I	Rat liver	5′-*p*-Fluorosulphonylbenzoyl-adenosine		Affinity labelling	146
Carboxylesterase	Rat liver	Amino acid phosphorodithioates, *N*-chloroacetyl amino acid esters		Irreversible inhibition	147
Carboxylic ester hydrolase	Human pancreas	Butane-2,3-dione	Arg	Two essential Arg groups	148
Carboxypeptidase C_u	*Citrus unshiu* MARC	DFP	Ser	Active-site Ser	149
Carboxypeptidase C_{ua} and C_{ub}	*Citrus unshiu* MARC	Methylene blue/*hν*	His	Active-site His	150
Cariotoxin	*Naja haje annulifera*	Cyclohexane-1,2-dione	Arg	Arg-36 not essential	151
Carnitine acetyl-transferase	Pigeon-breast muscle	Methoxycarbonyl-CoA disulphide	Cys	Reversible active-site modification	152
Casein kinase	Bovine lung	5′-[*p*-(Fluorosulphonyl)-benzoyl]adenosine		Catalytic subunit identified	153
Catechol-*O*-methyl-transferase	*E. coli* MRE 600	8-Azido-5-adenosyl-L-methionine		Analogue is substrate in the dark	154
Cathepsin B	Bovine spleen	Gly-Phe-cystamine 2-pyridyl disulphide	Cys	Active-site-directed disulphide exchange	155
Cathepsin B	Bovine spleen	Peptidyl diazomethyl ketones	Cys	Exploration of active site	156
Cathepsin B	Rat	[^{125}I]-Tyr-Ala-Lys-Arg-CH_2Cl	Cys	Protease identification method	157
Cathepsin D-II	Japanese-monkey lung	Diazoacetyl-DL-norleucine methyl ester		Characterization of pro-cathepsin D-II	158
Cation transporters	Mouse leukaemia cells	PCMB	Cys	Non-permeable reagent identifies basis of melphalan resistance	159

Cellulase	*Pencillium funiculosum*	Polyvinyl alcohol/EDC	$-CO_2H$	Soluble enzyme derivative	160
Chloroplast coupling factor I	*Chlamydomonas reinhardi*	DCCI	$-CO_2H$	Reacts with β-subunit	161
Chloroplast coupling factor I	Spinach	4-Azido-2-nitrophenyl phosphate		Photoaffinity labelling	162
Cholecystokinin and receptor	Mouse pancreas	Disuccinimidyl suberate	$-NH_2$	Characterization by crosslinking	163
Cholecystokinin receptor/cholecystokinin	Mouse pancreatic plasma membrane	Disuccinimidyl suberate		Receptor characterization	164
Cholinesterases	Human, horse	α,ω-Bis(aziridinium)alkanes		Kinetic comparisons	165

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Chromatophores	*Rhodospirillum rubrum*	4-Chloro-7-nitrobenzofurazan DCCI, EEDQ	Tyr $-CO_2H$	Labelling of β-subunit and F_1-ATPase	166, 167
Chymotrypsin		Acryloyl chloride, maleic anhydride	Lys	Heterogeneity assessed	168
Chymotrypsin		Haloenol lactones	Ser	Enzyme-activated inhibitors	169
Chymotrypsin		2,2,5,5-Tetramethyl-4-iodo-acetamide pyrrolidino-1-oxyl 5-doxylpalmitic acid		Spin-label study of enzyme in octane	170
Chymotrypsin	Bovine	Bis(4-fluorophenyl)carbamoyl chloride	Ser-195	^{19}F n.m.r. probe	171
Chymotrypsin	Bovine	α-Bromo-4-amino-3-nitro-acetophenone	Met-192 Asp-64	pH-Dependence of Asp modification	172
Chymotrypsin	Bovine	2-(2,4-Dinitrophenoxy)-2-oxo-*trans*-5,6-tetramethylene 1,2,3-dioxaphosphorinane	Ser-195	Stable phosphoryl enzyme	173
Chymotrypsin	Bovine	Methyl acetimidate	$-NH_2$	Effects on alkaline conformations	174
Citrate lyase complex	*Klebsiella aerogenes*	Phenylglyoxal, butane-2,3-dione	Arg	Both lyase and transferase activities inhibited	175
Citrate synthase	*Acinetobacter calcoaceticus*	Dithiobis-(succinimidyl propionate)	$(-NH_2)$	Activity restored upon reduction	176
Citrate synthase	*B. megaterium*	Dimethyl suberimidate, glutaraldehyde		Dimeric structure confirmed	177
α-Clostripain	*Clostridium histolyticum*	Iodo-[1-^{14}C]acetic acid	Cys	Active-site sequence	178
Coat protein	Alfalfa mosaic virus	*p*-Hydroxymercuriphenyl sulphonic acid	Cys-108, 169	Heavy-metal derivative for diffraction	179
Coat protein	Bacteriophage fd	Methyl [^{3}H]acetimidate	Lys-8	No loss of infectivity	180

Colipase	Porcine pancreas	Tetranitromethane	Tyr	Reaction in various micelles and water	181
Collagen	Rat-tail tendon	Pyrenesulphonylhydrazine, pyrenebutyrylhydrazine		Two adjacent aldehydes in monomeric young collagen	182
Collagenase	*Hypoderma lineatum*	[^{3}H]DFP	Ser	Active-site region sequenced	183
Complement C2 and Factor B	Human plasma	PCMB, *m*-chloroperbenzoic acid, iodine	Cys	Oxidation enhances haemolytic activity	184
Complement C3		[^{14}C]Methylamine	Glu	Thiol ester identified	185

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Complement C3	Human	Sodium borohydride	β-Cys-γ-Glu bond	Internal thio-ester reduced	186
Complement C3b	Human	Dimethyl suberimidate	Lys	Dimer binds receptor more strongly	187
Complement C3/ erythrocytes	Human/sheep	SPDP	$-NH_2$, Cys	Cytolytic assays	188
Complement complexes	Human	[^{125}I]Hexanoxydi-iodo-*N*-(4-azido-2-nitrophenyl)-tyramine		Membrane insertion of subunits identified	189
Corticosteroid-binding globulin	Human	Phenylglyoxal	Arg	Activity depends on single Arg	190
Creatine kinase	Rabbit-heart mitochondrial membrane	Various —SH reagents	Cys	Negatively charged organomercurials solubilize	191
Creatine kinase	Rabbit muscle	ADP and ATP 2′,3′-dialdehydes	Lys	Reagents phosphorylated by phosphocreatine	192
Creatine kinase	Rabbit muscle	Glutaraldehyde	$-NH_2$	Denaturation and re-assembly	193
Cutinase	*Fusarium solani*	[^{3}H]DFP [^{3}H]Iodoacetamide	Ser Cys	Active-centre peptide not homologous with other proteases	194
Cyclic nucleotide phosphodiesterase	Mutant S49 lymphoma	[^{32}P]cGMP, [^{32}P]cAMP, u.v.		Photoaffinity labelling	195
γ-Cystathionase	Rat liver	Trifluoromethionine	($-NH_2$)	Acts by releasing CSF_2	196
Cysteine proteases	*Carica papaya, Actinidia chinensis*	4,4′-Dipyridyl disulphide. DTNB	Cys	Neutral and anionic active-centre probes	197
Cysteine sulphinate decarboxylase	Rat liver	β-Methylene DL-aspartate		Efficient inactivation *in vivo*	198

Cytochrome b_5	Rabbit liver	Diazirino and diazo photo-activatable phospholipids		Photoaffinity labelling of transferable and non-transferable forms	199, 200
Cytochrome b–c_1 complex	Rat-liver mitochondria	DCCI	$-CO_2H$, $-NH_2$	Proton translocation inhibited	201
Cytochrome b–c_2 oxidoreductase	*Rhodopseudomonas sphaeroides*	DCCI		Irreversible inhibition	202
Cytochrome b_6-f complex	Spinach chloroplast	2-Azido-2′,4,4′-trinitro-6-(s-butyl)diphenylether/$h\nu$		B_6 and Rieske Fe—S peptide subunit labelled	203
Cytochrome c	Horse	*O*-Methyl isourea and others	Lys	^1H and ^{13}C n.m.r. study	204
Cytochrome c	Horse	Pentane-2,4-dione, cyclohexane-1,2-dione, camphorquinone-10-sulphonic acid	Arg	Arg-91 and Arg-38 modified	205

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Cytochrome *c*	Horse heart	4-Fluoro-3-nitrophenylazide	Lys-13	Activation for cross-linking to cytochrome c_1	206
Cytochrome *c*	Horse heart	*m*-Trifluoromethylphenyl isocyanate 4-Chloro-3,5-dinitrobenzoate	Lys-72	Comparison with enzymatic modification	207
Cytochrome *c*, β-galactosidase, immunoglobulin G	Horse heart, *E. coli*	CNBr-treated lipopolysaccharide		Reactive cyanate derivative	208
Cytochrome *c*/ubiquinone-cytochrome *c* oxidoreductase	Bovine	EDC, CMC	$-CO_2H$	Some crosslinking observed	209
Cytochrome *c* oxidase	Bovine heart	1-Ethyl-3-{3-[^{14}C](trimethylamino)propyl}carbodi-imide	$-CO_2H$	Cytochrome *c* binding site modified	210
Cytochrome *c* oxidase	Yeast	3-Azido-2,7-naphthalene disulphonate 5-azido-1-naphthalene sulphonate		Hydrophilic and hydrophobic domains labelled	211
Cytochrome *f*/plastocyanin	Turnip/spinach	EDC	$-CO_2H$	Zero-length crosslinking	212
Cytochrome P450	Rat-liver microsome	Acetic anhydride	Lys	Role of Lys in hydroxylation	213
Cytochrome P450/NADPH reductase	Porcine liver	Monobromobimane	—SH	Single essential Cys	214
Cytochrome $P450_{scc}$		20-(1-Propynyl/1-hexynyl/hexa-1,5-dinyl)-5-pregnen-3β,20α-diol		Suicide inhibitors	215
Cytochrome P450-LM2	Rabbit	FITC	N-Terminal Met	Distance to haem iron estimated	216
Cytochrome P450-LM2	Rabbit liver	FITC		Energy-transfer distances measured	217

Cytochrome P450-LM2	Rabbit liver	Tetranitromethane	Tyr	Tyr is sixth Fe ligand	218
Cytosolic protein	Rat liver	7-Hydroxymethyl-12-methyl-benz[a]anthracene		*Via* 7-HMBA sulphate	219
Dehydrogenases	Various	5-AMP ω-haloalkyl esters		Potential active-site-directed reagents	220
Diamine oxidase	*Euphorbia characias* latex	4,4'-Dithiodipyridine	Cys	Cys reactive in presence of substrate	221
Dihydrofolate reductase	*Streptococcus faecium*	Iodoacetate	Met	~ 30% of Met residues modified	222
Diphtheria toxin	*C. diphtheriae*	Pyro-Glu-His-Pro-amide/iodo-acetylcystamine, SPDP	(Lys)	Hydrophobic demain of toxin targeted to pituitary	223
DNA polymerase	*E. coli*	Sulphydryl reagents	Cys	Essential thioredoxin SH	224

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
DNA topoisomerase	*Micrococcus luteus*	Tetranitromethane	Tyr	Inactivation	225
Dopamine-β-hydroxylase	Bovine adrenals	Bleomycin		Light-dependent inactivation	226
Dopamine-β-hydroxylase	Bovine	1-Phenyl-1-aminomethylethene		Turnover gives 2,3-dihydroxy-2-phenylpropylamine	227
Early cysteine-labelled protein	*Tritium aestivum*	Iodo[^{14}C]acetamide	Cys	Detection of novel wheat-germ protein	228
EGF receptor	Human carcinoma A431	5′-*p*-Fluorosulphonylbenzoyl [8-^{14}C]adenosine		Protein kinase and receptor on same chain	229
Elastase	Porcine pancreas	2-*O*-Methoxy-polyethylene glycol-4,6-dichloro-*s*-triazine	Lys	Elastolytic activity reduced	230
Electron-transfer flavoprotein	Porcine liver	TNBS, acetic anhydride, methyl acetimidate	Lys	Kinetic study	231
Elongation factor 2	Rat liver	NEM, TNBS	Cys, $-NH_2$	Study of essential groups	232
Elongation factor G	*E. coli*	Di-epoxybutane		Crosslink to 23S RNA of 70S ribosome	233
Elongation factor Tu	*E. coli*	6-(2-Nitro-4-azidophenylamino)-caproate/*hν*		Photoaffinity crosslinking to Phe tRNA	234
Enkephalin convertase	Bovine pituitary	Bromoacetyl-D-arginine		Irreversible inhibition	235
Epidermal growth-factor-like transforming growth factor/EGF receptor	Rat embryo cells and human A431 carcinoma cells	Disuccinimidyl suberate	$-NH_2$	Receptor characterization	236
Esterase A	Rat urine	PMSF, D-Phe-Phe-Arg-CH_2Cl	Ser, His	Affinity labelling	237
Exotoxin A	*Pseudomonas aeruginosa*	Azido analogues of NAD		Toxoid preparation by photoaffinity labelling	238
Fatty-acid synthetase	Pigeon liver	*S*-(4-Bromo-2,3-dioxobutyl)-coenzyme A	Cys	3-Oxoacyl synthetase activity inhibited	239

Fatty-acid synthetase	Chicken liver	Dibromopropanone, *o*-phthalaldehyde	Cys, Lys, Cys	Study of β-ketoacyl synthetase site	240
Fatty-acid synthetase	Chicken liver	DTNB, iodoacetamide	Cys	Intrasubunit disulphides formed	241
Fatty-acid synthetase	Chicken liver	4-(1-Pyrenyl)butyryl-CoA		Fluorescence study	242
Fatty-acid synthetase	Chicken liver	Pyridoxal 5-phosphate/$NaBH_4$	$-NH_2$	Active-centre mapping	243
Fatty-acid synthetase	Pig liver	[^{3}H]Tetrahydrocerulenin	Cys	Affinity label	244
Ferredoxin	Spinach	DEP	His	Activity lost when second His modified	245
Ferredoxin II	*Anabaena variabilis*	TNBS	Lys	N.m.r. assignment aid	246

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Ferredoxin	Spinach	TNBS	Lys	Electron transport inhibited	247
Ferritin	Human	Glutaraldehyde		IgG conjugate allows cell selection	248
Fibrin	Bovine, human	Glutaraldehyde	($-NH_2$)	Electron-microscopy study	249
Fibrinogen	Human	Photo-oxidation	His	Bβ-16 essential for polymerization	250
Fibrinogen	Human	Various anhydrides, *O*-methylisourea, ethyl acetimidate	Lys	Critical for plasminogen activator stimulation	251
Fibronectin	Hamster plasma	2-Nitro-5-thiocyanobenzoic acid	Cys	Domains aligned	252
Fibronectin	Human plasma	Succinic anhydride, butane-2,3-dione	Lys, Arg	Arg essential for gelatin binding	253
Flavocytochrome b_2	Yeast	2-Keto-3-butynoic acid	Cys	Three modified Cys spatially close	254
Formate dehydrogenase	*Achromobacter parvulus*	Pyridoxal, pyridoxal phosphate, formaldehyde	Lys	Inactivation	255
Formate dehydrogenase	*Achromobacter parvulus*	Woodward's reagent K	$-CO_2H$	One essential $-CO_2H$	256
N-Formyl peptide receptor	Human leukocyte	*N*-Formyl-*N*-Leu-Leu-Phe-*N*-Leu-Tyr-N^{ϵ}-6-(4′-azido-2′-nitrophenylamino)hexanoyl-lysine		Labelling of cell-stimulation site	257
Fructose 1,6-diphosphatase	Porcine kidney	2-Azido-AMP		Inhibition, photoaffinity labelling	258
Fumarase	Porcine	Tetranitromethane	Tyr	Inactivation	259
GABA receptor	Rat cerebellum synaptosome	[^{3}H]Muscimol/u.v.		Photoaffinity labelling	260
Gag precursor Pr 65	Gadzar murine sarcoma virus	Dimethyl suberimidate, phenylene dimaleimide, difluorodinitrobenzene		Lipid–protein interactions studied	261

Galactose-transport protein	*E. coli*	*N*-([2-^{3}H]Ethyl)maleimide	(Cys)	37K protein identified	262
α-D-Galactosidase	Green coffee beans	CNBr-activated dextran T70	($-NH_2$)	K_m and K_s lowered	263
β-Galactosidase	Bovine testis	CNBr-activated dextran, acetylated dextran, and methylated dextran		Effect on thermal and proteolytic stability	264, 265
Gene 5 protein	Bacteriophage f1	[^{3}H]$BrCH_2CONH(CH_2)_2NH$-(5′-*N*)-d(pT-C-T-A-G)		Affinity labelling	266
Glucagon		Nitromalondialdehyde	Arg	Forms δ-(5-nitro-2-pyrimidyl)ornithine at high pH	267

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Glucagon		TNBS	Lys-12	Effects on receptor binding, *etc.*	268
β-1,3-Glucanase	*S. sachalinensis*	2′,3′-Epoxypropyl-1-thio-β-D-glucopyranoside		Affinity labelling	269
β-1,3-Glucanase	*S. sachalinensis*	Pentane-2,4-dione, TNBS	Lys	Kinetics unchanged	270
Glucoamylase	*Aspergillus awamori*	(i) EDC, taurine (ii) NBS	(i) $-CO_2H$ (ii) Trp	Active-site residues modified	271
Glucoamylase	*Rhizopus niveus*	NBS	Trp	Kinetic discrimination	272
Glucocorticoid receptor	Mouse	[^{3}H]Dexamethasone mesylate	Cys	Affinity labelling	273
Glucocorticoid receptor	Rat liver	Dexamethasone 21-mesylate		Receptor activated in intact cells	274
Glucocorticoid receptor	Rat thymocyte, human lymphoid	[^{3}H]Dexamethasone 21-mesylate		Receptor characterization	275
Glucocorticoid receptor		[^{3}H]Triamcinolone acetonide		Photoaffinity labelling	276
Glucose-transport protein/[^{3}H]cyto-chalasin B	Rat adipocyte	Hydroxysuccinimidyl azidobenzoate		Photoaffinity crosslinking	277
Glucose dehydrogenase	*Bacillus megaterium*	Bromoacetylpyridine	His, (Lys)	Active-site modification	278
Glucose dehydrogenase	*Bacillus megaterium*	Methyl [2-(5′-dansylamido)-methylimidate]	Lys	Active-site Lys labelled	279
Glutamate decarboxylase	*E. coli*	Butane-2,3-dione	Arg	One essential Arg	280
Glutamate dehydrogenase	Bovine	5′-[*p*-(Fluorosulphonyl)benzoyl]-1,N^6-ethenoadenosine		GTP site 18 Å from ATP site	281
Glutamate synthase	*E. coli*	Phenylglyoxal, pyridoxal 5′-phosphate	Arg, Lys	Ligand-binding studies	282
Glutamine-binding protein	*E. coli*	(i) DEP (ii) NBS	(i) His (ii) Trp	Inhibited restoration of glutamine transport	283

Glutamine phospho-ribosyl pyrophosphate amidotransferase	*Bacillus subtilis*	6-Diazo-5-oxo-L-norleucine	Cys	Role of 4Fe–4S centre	284
Glutamine synthetase	*E. coli*	Ascorbic acid/O_2	His	Model for *in vivo* degradation	285
Glutamine synthetase	Pea chloroplasts	Methylene Blue/*hv*, PCMB	His, Cys	Inhibition	286
γ-Glutamyltransferase	Rat	L-(α*S*,5*S*)-α-Amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid, phenylglyoxal	Arg	Arg binds anions	287
Glutathione reductase	Yeast	*N*,*N*′-Bis-(4-azidobenzoyl)-glutathione disulphide		Partial photoinactivation	288

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
γ-Glutamyltransferase	Rat-liver canalicular membrane vesicles	L-(α*S*,5*S*)-α-Amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid		Affinity labelling	289
γ-Glutamyltransferase	Bovine	Butane-2,3-dione	Arg	Essential Arg	290
Glutathione transferase	Rat-liver microsomes	NEM	Cys	Characterization of activation	291
Glyceraldehyde 3-phosphate dehydrogenase	Yeast, rat, rabbit	Butane-2,3-dione	Arg	Inactivation	292, 293
D-Glyceraldehyde-3-phosphate dehydrogenase	*Palinurus versicolor*	(i) Active-site carboxymethylation (ii) NAD^+/u.v.		NAD^+ covalently linked	294
D-Glyceraldehyde 3-phosphate dehydrogenase	Rabbit muscle, yeast	Iodoacetate/$h\nu$	Cys	Photoinduced formation of fluorescent addent with NAD^+	295
Glyceraldehyde 3-phosphate dehydrogenase	Rabbit	Pyridoxal 5′-phosphate	Lys-191, -212	Role of erythrocyte binding	296
Glycerol 3-phosphate dehydrogenase	Rabbit skeletal muscle	FITC		Study of fructose 1,6-bisphosphate aldolase complex	297
Glycine receptor	Rat spinal cord	[^{3}H]Strychnine/$h\nu$		Receptor study	298
Glycogen phosphorylase	Muscle	Sodium periodate	Cys	Slow oxidation inactivates	299
Glycogen synthase 1	Bovine heart	Oxidized glutathione	Cys	Specific disulphide interchange	300
Glycoproteins	Mouse thymocytes	Lucifer Yellow CH/sodium periodate		New fluorescent-labelling technique	301
Glycoprotein G	Vesicular stomatitis virus	ω-[9-^{3}H]Diazirinophenoxy nonanoate		Viral phospholipids labelled as well	302

Glyoxylase I	Yeast	Phenylglyoxal, butane-2,3-dione, camphorquinone 10-sulphonic acid	Arg	Inactivation	303
Gonadotropin-releasing hormone receptor	Rat pituitary cells	[Azidobenzoyl-D-Lys^6]GnRH		Photoaffinity labelling	304
Gonadotropin-releasing hormone receptor	Rat	[D-pGlu^1,D-Phe^2,D-Trp^3,D-Lys^6-(azidobenzoyl)]-gonadotropin-releasing hormone		M_r 60K protein labelled	305
Gramicidin S synthetase	*Bacillus brevis* ATCC 9999	D-Phe-Pro-Val	Cys	Stable thioester intermediate	306
Gramicidin S synthetase 2	*Bacillus brevis* ATCC 9999	L-Phenylalanine chloromethyl ketone		Non-specific inhibitor	307
Growth hormone	Bovine	Cyclohexane-1,2-dione	Arg	Only one Arg not reactive	308

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Growth hormone	Bovine	EDC/glycine methyl ester	$-CO_2H$	Hydrolysis of ester restores activity	309
Growth hormone/ hepatocyte receptor	Human/rat	Disuccinimidyl suberate		M_r 100K labelled	310
GTP-binding protein	Human platelets	5′-*p*-Fluorosulphonylbenzoyl guanosine		Affinity labelling	311
L-Gulonolactone oxidase/ antibody	Rat, chicken/guinea-pig	Glutaraldehyde	$(-NH_2)$	Immunoprecipitate detoxified in guinea-pig	312
Haemocyanin	Lobster	Acetic anhydride	Lys	Subunit-structure study	313
Haemoglobin	Human	Glycolaldehyde/glyceraldehyde and $Na(CN)BH_3$	α-NH_2	Effects on O_2 affinity	314
Haemoglobin	Human	Glyceraldehyde	α-NH_2	Amadori rearrangement to ketoamine adduct	315
Haemoglobin (apo)	Human	Pyridoxamine 5′-phosphate	$Val(NH_2)$	Dimensions in solution	316
Haemoglobin	Human	Bis-(3,5-dibromosalicyl) fumarate	Lys-82β His-146β	Intramolecular cross-linking	317
Haemoglobin	Human	Glyoxalate/$Na(CN)BH_3$	α-NH_2	Affects O_2 affinity	318
Haemoglobin	Human	Periodate-oxidized dextran	Lys	Decreased clearance	319
Haemoglobin	Human	Pyridoxal 5′-phosphate, succinylated inulin oxy-succinimide ester	(Lys)	Blood substitute	320
Haemoglobin-CO	Human	Methylphenylmercury	Cys-G11α, -G14β	Normally inaccessible residues	321
Haemoglobin S	Human	Bifunctional imidoesters	Lys	Aggregation retained	322
Haemoglobin S	Human	Glycine ethyl ester/EDC	Glu-43β	Highly specific	323
High-density lipoproteins	Human	Dimethyl 3,3′-dithiobispropion-imidate, 1,5-difluoro-2,4-dinitrobenzene	Lys	Quaternary-structure study	324

High-mobility-group protein 1, histone oligomers		Dimethyl suberimidate, dimethyl 3,3′-dithiobis-propionimidate, EDC	$-NH_2$, $-CO_2H$	Study of HMG-histone interactions	325
Histidine decarboxylase	Rat gastric mucosa	α-Fluoromethyl-histidine			326
Histidine decarboxylase	Rat mast cell	α-Fluoromethyl-histidine		Reduces His uptake	327
Histone H1	Rat-liver nuclei	[^{14}C]Acetyl-Co A	$-NH_2$	Non-enzymatic labelling	328
Histones H1, H1°	Mouse-liver nuclei	Dimethyl 3,3′-dithiobis-propionimidate		Study of H1° neighbours in chromatin	329
Histone octamers	Chicken erythrocyte	Dimethyl suberimidate	$-NH_2$	Subunit assembly	330

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Histone H1	Bovine kidney	Dimethyl suberimidate, dimethyl dithiobispropionimidate	$-NH_2$	Close-contact study	331
		EDC	$-CO_2H$		
Histone H1	Calf thymus, bovine liver	(i) 3-(5-Fluoro-2,4-dinitroanilino)-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl	(i) Lys	Spin-label study of DNA interaction	332
		(ii) *N*-(2,2,5,5-Tetramethyl-3-carbonylpyrrolidinyl-1-oxyl) imidazole	(ii) Tyr		
Histone H1	Calf thymus nuclei	Methyl 4-mercaptobutyrimidate	$-NH_2$, Cys	Chromatin structural study	333
Histone kinase	Porcine brain	(i) Tetranitromethane/reduction	(i) Tyr-72	Insertion of spectroscopic probes	334
		(ii) Dansyl chloride or spin label	(ii) Tyr-72 (NH_2)		
D-β-Hydroxybutyrate dehydrogenase		Cyclohexane-1,2-dione	Arg	Single essential Arg	335
3α,20β-Hydroxysteroid dehydrogenase	*Streptomyces hydrogenans*	5′-Bromoacetamido-5′-deoxyadenosine		Label for nucleotide-binding sites	336
17β,20α-Hydroxysteroid dehydrogenase	Human placenta	16-Methylene estradiol-17β		Suicide inactivation	337
Immunoglobulins, cytotoxic agents	Various	Various crosslinkers			338
Immunoglobulin E	Mouse	FITC, *N*-{4-[7-(diethylamino)-4-methylcoumarin-3-yl]phenyl} maleimide	Lys, Cys	Membrane-receptor complex distance mapping	339
Immunoglobulin G	Human, rabbit	Dithiobis-(succinimidyl) propionate	Lys	Monocyte C2 production stimulated	340

Immunoglobulin G	Rabbit	*N*-(7-Dimethylamino-4-methyl-coumarinyl) maleimide	Cys	Fluorescence probe	341
Immunoglobulin G	Rabbit	Biotin *N*-hydroxysuccinimide ester		Antibody labelling of red cells *via* avidin	342
Immunoglobulin G	Rabbit	*N*-Succinimidyl-6-(4′-azido-2-nitrophenylamino) hexanoate	($-NH_2$)	Preparation of IgG–antibody conjugates	343
Immunoglobulin G	Mouse hybridoma	*N*-Hydroxysuccinimide ester of palmitic acid	($-NH_2$)	Formation of IgG-labelled liposomes	344
Immunoglobulin G, liposomes		SPDP	($-NH_2$, Cys)	Antibody-targeted liposomes	345
Immunoglobulin light chains, antigen E	Ragweed	4-Dimethylaminoazobenzene 4′-iodoacetamide, 4-dimethylaminobenzene 4′-*N*-maleimide	Cys	Reversed-phase h.p.l.c. of hydrophobic labelled peptides	346
Immunoglobulin M	Mouse B lymphocytes	4,4′-Diphenyldiazonium disulphide fluoroborate		Membrane-associated proteins	347

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Immunoglobulin M		*N*-(*m*-Maleimidobenzoyl)-dipalmitoylphosphatidyl-ethanolamine		Liposome targeting	348
Initiation factors 1, 2, and 3	*E. coli*	2-Iminothiolane	Lys	Crosslinks in complexes with 30S ribosomal subunits	349
Initiation factor 3 (40 S subunit)	Rabbit reticulocyte	Iminothiolane, H_2O_2	$-NH_2$	Disulphide formation	350
Insulin	Bovine	SPDP, dithiothreitol	$-NH_2$	Linked to phosphatidyl ethanolamine in liposomes	351
Insulin	Bovine	Malonyl bis(methionine 4-nitrophenyl ester), oxalyl bis-(methionyl 4-nitrophenyl ester)	$-NH_2$	Reversible crosslinking	352
Insulin	Bovine	DnsCl	B1-NH_2	Change in aggregation state	353
Insulin	Bovine pancreas	2-Iodo-*N*-methylpyridinium iodide	Tyr	Activity reduced	354
Insulin	Porcine	D_2O, pD 13	Cys_2	β-Elimination gives dehydroamino acid, n.m.r. study	355
Insulin	Porcine	OH^-, DTNB	Cys_2	Alkaline fission mechanistic study	356
Insulin	Porcine	$HCNO/Na(CN)BH_3$	$-NH_2$	50% activity retained	357
Insulin	Porcine	$Na^{125}I$, lactoperoxidase	Tyr	Binding to receptor of four monoiodoinsulins	358
Insulin/insulin receptor		Disuccinimidyl suberate	$(-NH_2)$	Receptor characterization	359

Insulin/ricin B-chain	Porcine/*Ricinus communis*	SPDP	$-NH_2$, Cys	Receptor-binding study	360
Insulin receptors	Human adipocyte	Photolabile derivatives of insulin		Insulin-receptor complex metabolism studied	361
Insulin receptor	Rat	N^{ϵ}-B_{29}-(2-Nitro-4-azidophenyl-acetyl) insulin		138K receptor internalized	362
Insulin receptor	Rat adipocyte	DTNB	Cys	Receptor disulphide exchange	363
Insulin receptor	Rat hepatocyte	[α-^{32}P]ATP 2',3'-dialdehyde		Receptor kinase activity	364
Insulin receptor, insulin	Turkey erythrocyte, porcine	Disuccinimidyl suberate, glutaraldehyde	$-NH_2$	Subunit-structure characterization	365
Insulin receptor protein kinase		Disuccinimidyl suberate	$-NH_2$	Activity study	366
		NEM	Cys		

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Ion transporter(s)	Human lymphocyte	NEM	Cys	Inhibition of cell-volume changes	367
Isocitrate dehydrogenase	Porcine heart	2′,3′-Dialdehyde derivatives of TPNH and TPN		Affinity labelling	368
Isocitrate dehydrogenase (NAD-dependent)	Porcine heart	2′,3′-Dialdehyde derivative of ADP	Lys	Inactivation, loss of PP_i	369
2-Ketoglutarate and pyruvate dehydrogenase complexes	Bovine heart	Methylene cyclopropyl pyruvate		Formation of methylene-cyclopropylacetyl-CoA	370
Δ^5-3-Ketosteroid isomerase	*Pseudomonas testosteroni*	Light-activatable, agarose-bound steroid		Solid-phase photoaffinity label	371
Δ^5–3-Ketosteroid isomerase	*Pseudomonas testosteroni*	Spiro-17β-oxiranyl-Δ^4-androsten-3-one, spiro-17β-oxiranyloestra-1,3,5(10),6,8-pentaene-3-ol	Asp-38	Enzyme may bind steroids in two ways	372
Kininogen, high mol. wt.	Human plasma	5-(Iodoacetamido)fluorescein	Cys	Fluorescence study of contract activation	373
lac carrier protein	*E. coli* T206	*p*-Nitro[2-^{3}H]phenyl-α-D-galacto-pyranoside		Membrane topology	374
Hβ-Lactamase I	*Bacillus cereus* 569	6-β-(Trifluoromethane sulphonyl)amido penicillanic acid sulphone (and *N*-methyl derivative)	Ser-70	Inactivation	375
β-Lactamase	*E. coli* TEM-1	6-Acetylmethylene penicillanic acid	(Ser)	Stoicheiometric inactivation	376, 377
β-Lactamase	*Staphylococcus aureus* PC 1	Sodium 3-dansylamidomethyl-7-β-(thienyl-2′)-acetamido ceph-3-em-4-oate	Ser	Covalent intermediate trapped	378
L-Lactate dehydro-genase	*Lactobacillus casei*	3-Bromopyruvate	His-188	Affinity labelling of allosteric site	379

Lactate dehydrogenase	Porcine heart	Bicyclo[4,1,0]heptan-7-ylmethanol		Inactivation *via* cyclopropane ring opening	380
Lactate dehydrogenase/alcohol dehydrogenase	Bovine heart/horse liver	Glutaraldehyde	($-NH_2$)	Site-to-site directed co-immobilization	381
Lactogenic binding sites, [^{125}I]-somatotropin	Rat liver	Dimethyl suberimidate, disuccinimidyl suberate, EDC, *p*-azidophenacyl bromide		63K and 96K receptor species	382
Lactogenic and somatogenic hormone-binding sites	Rabbit mammary gland, rabbit liver	Ethyl-4-azidophenyl 1,2-dithiobutyrimidate-modified human growth hormone		Monomeric and multimeric binding sites	383
β-Lactoglobin B		$MeCH_2CH_2SH$, $NH_2CH_2CH_2SH$, $HOOCCH_2CH_2SH$, $HOCH_2CH_2SH$	Cys	Conformational-stability study	384

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
β-Lactoglobulin	Bovine	Phosphorus oxychloride		13 mol mol^{-1} substituted	385
Lactose carrier	*E. coli*	(i) DEP (ii) Phenylarsinoxide, plumbagin	His Cys	Galactoside binding affected	386
Lactose synthase	Bovine milk	4-Azido-2-nitrophenyl uridylyl pyrophosphate		Photoaffinity labelling	387
Leucyl-tRNA synthetase	*Euglena gracilis*	ATP γ-(*p*-azidoanilidate)		Photoaffinity labelling	388
Low-density lipoprotein	Human plasma	Glucose	Lys	Forms Schiff base	389
Luciferase	*Vibrio harveyi*	1-Diazo-2-oxo-undecane		Photoaffinity labelling of aldehyde-binding site	390
Lysozyme	Hen egg	EDC	Leu-129 (—COOH), Lys-13	Intramolecular crosslink, imidazole catalysis	391
Lysozyme	Hen egg	*N*-Acetoxy-*N*-acetyl-2-aminofluorene, DNA		Carcinogenicity model	392
Lysozyme	Hen egg	2-Methylpropanal/O_2/peroxidase, riboflavin		Flavin activation by triplet acetone	393
Lysyl oxidase	Bovine aorta	β-Aminopropionitrile		Ketenimine intermediate postulated	394
α_2-Macroglobulin	Human	IAEDANS	Cys	Energy transfer between inhibitor and labelled protease	395
Malic enzyme	Pigeon liver	2-Nitro-5-thiocyanobenzoic acid, 2,4-dinitrophenylthiocyanate	Cys	Cyano enzyme active, atypical reactions	396
Membrane proteins	*Fasciola hepatica*	Lysergic acid diethylamide/$h\nu$		Possible photoaffinity label	397
Membrane proteins	Human erythrocyte	7-Azidofluphenazine, 7-azidotrifluprom azine methiodide		Evidence for lipid solubility of phenothiazines	398

Membrane proteins	Human erythrocyte	[^{14}C]-4-Benzamido-4′-isothiocyanostilbene 2,2′-disulphonate		Affinity labelling of band 3	399
Membrane proteins	Human erythrocyte	*N*-Maleoylmethionine [^{35}S]-sulphone	Cys	Two —SH proteins not labelled in Rh_{null} cells	400
Membrane proteins	Human erythrocyte	Various lipophilic, spin-labelled bifunctional crosslinking agents			401, 402
Membrane proteins	Mouse brain	[20-^{3}H]Phorbol 12-*p*-azidobenzoate 13-benzoate		Low specificity for proteins	403
Membrane proteins	Rat-liver mitochondria	*N*-(1-Oxyl-2,2,5,5-tetramethyl-3-pyrrolidinyl) maleimide	Cys	E.s.r. study	404

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Mevalonate 5-pyrophosphate decarboxylase	Chicken liver	Phenylglyoxal	Arg	Essential Arg	405
β_2-Microglobin	Human urine	$Na^{125}I$/iodogen	Tyr	Sites located, 4^y structure of HLA	406
Microsomal proteins	Rat liver	[6,7-^{3}H]-17α-Ethinyloestradiol		Very low uptake	407
Monoamine oxidase	Bovine liver	*trans*-2-Phenylcyclopropylamine	Cys	New mechanism proposed	408
Monoamine oxidase, type A	Rat	*N*-[1-(*o*-Iodophenoxy)ethyl]-cyclopropylamine·HCl	Cys	Cyclopropanone imine intermediate postulated	409
Monoamine oxidase, type B	Rat liver	3-[4-(3-Chlorophenyl/methoxy)-phenyl]-*S*-(methylamino/methyl)-2-oxazolidinone-methanesulphonate (MD 780236)		Enzyme-activated irreversible inhibition	410
Monoclonal antibody M-2K^k	Murine hybridoma	*N*-Succinimidyl palmitate		Preparation of immunoliposomes	411
Muscarinic receptor	Chick heart	NEM	Cys	Potentiation of cation effects on ligand binding	412
Muscarinic receptors	Rat brain and heart	*N*-Methyl-4-piperidyl *p*-azidobenzilate		Oligomeric structure found	413
Myosin	Rabbit	CMC, nitrotyrosine ethyl ester	$-CO_2H$	Essential carboxyl located	414
Myosin	Rabbit	EDC	$-CO_2H$	Alkali light chain cross-linking with actin	415
Myosin	Rabbit	*N*-(Iodoacetyl)-*N'*-(5-sulpho-1-naphthyl)ethylenediamine	Cys	Cys-177 *ca.* 5.7 Å from active site	416
Myosin	Rabbit	[^{3}H]-1,5-IAEDANS, *N*-[4-(dimethylamino)-3,5-dinitrophenyl] maleimide	Cys	Inter-thiol distances	417

Myosin	Rabbit	1,5-IAEDANS, 5-(iodoacetamido)fluorescein		Energy-transfer study	418
Myosin	Scallop	DTNB, TNBS	Lys	Effect of regulatory light chain	419
Myosin light chains	Scallop, rabbit	(i) *p*-Azidophenacyl bromide (ii) Benzophenone 4-maleimide (iii) 4-Azidobenzoic acid *N*-oxysuccimidyl ester	(i) Cys (ii) Cys (iii) Lys	Linking to N-terminal region blocks Ca^{2+}-ATPase action	420
Myosin subfragment 1		*N,N'-p*-Phenylene dimaleimide	Cys	Crosslinking traps ADP	421
Myosin subfragment 1	Rabbit muscle	EDC	Lys, —COOH	Mapping of actin-binding sites	422
Myosin subfragment 1	Rabbit muscle	TNBS	Lys	ATPase mechanism	423

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Myosin subfragment 1, actin	Rabbit	*N*-(Bromoacetyl)-*N'*-(1-sulpho-5-naphthyl)ethylenediamine, 5-(iodoacetamido)fluorescein	Cys	Fluorescence-energy transfer in complex	424
Myosin, albumin	Rabbit skeletal muscle, bovine serum	Thiols/Ce^{4+}	Cys	Rigid label *via* spin-trapping	425
Na^+ channel	*Electrophorus electricus*	[^{3}H]Ethylenediamine tetrodotoxin, disuccinimidyl suberate		270K polypeptide labelled	426
NADP malate dehydrogenase	Maize	Procion rubine MX-B		Label specific for active, reduced form	427
NADPH oxidase	Guinea-pig neutrophil	NEM	Cys	Inhibits O_2^- production	428
Nerve-growth-factor receptor	Human melanoma	Azidobenzoyl-[^{125}I]nerve growth factor		Receptor/factor complex modified then photolysed	429
Nerve-growth-factor receptor/nerve growth factor (^{125}I)	Rat pheochromocytoma/human melanoma	EDC, hydroxysuccinimidyl *p*-azidobenzoate		Receptor affinity-purification	430
Neurotoxic esterase	Various	[^{3}H]DFP	(Ser)	Intramolecular alkyl group transfer on 'ageing'	431
Neurotoxin A	*Clostridium botulinum*	Methylene Blue, *hν*	His, Trp	Essential Trp	432
Neurotoxins	Scorpion	Succinimidyl-6-(4'-azido-2'-nitrophenylamino) hexanoate		Photolabel for Na^+ channels	433
Neutral protease, Ca^{2+}-activated	Chicken muscle	Epoxysuccinyl derivatives, iodoacetic acid	Cys	Inactivation, effect of Ca^{2+}	434
Neutral protease, Ca^{2+}-activated	Chicken muscle	Iodoacetic acid	Cys	Active-site sequencing	435
Nicotinamide nucleotide transhydrogenase	Rat liver	(i) DCCI (ii) Butane-2,3-dione	(i) ($-CO_2H$) (ii) (Arg)	Inactivation	436

Nicotinic acetylcholine receptor	*Homarus americanus*	4-(*N*-Maleimido)benzyltrimethylammonium		α-Subunit, M_r 50 K, labelled	437
Nitrogenase Fe-protein	*Azotobacter vinelandii*	Iodo[2-^{14}C]acetic acid	Cys	Study of 14 Cys residues	438
Non-histone proteins/DNA	Mouse L 1210 cell nuclei	*cis*-Pt^{II}	Cys	Histone proteins not crosslinked	439
Nucleoside transporter	Human erythrocyte	N^6-(*p*-Azidobenzoyl)adenosine, nitrobenzyl thioinosine		Selective labelling of band 4.5 polypeptide	440
Nucleoside transporter	Human and porcine erythrocytes	[^{3}H]Nitrobenzyl thioinosine		Photoaffinity labelling of band 4.5 region	441
Oestradiol 17β-dehydrogenase	Human placenta	3-Bromoacetoxyoestrone, 12β-bromoacetoxy-4-oestrene-3,17-dione	His	Affinity labelling	442

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
17β-Oestradiol dehydrogenase/20-hydroxysteroid dehydrogenase	Human placenta	6β-Bromoacetoxyprogesterone		Both activities at single active site	443
Oestradiol 17β-dehydrogenase	Human placenta	16-Oxo-oestrone	Arg	Affinity labelling of two essential Arg	444
Oestrogen receptor	Calf uterus	Cyclohexane-1,2-dione	Arg	Role of Arg in receptor activation	445
Oestrogen receptor	MCF-7 cells	3-Hydroxy-17β-(*p*-nitrophenyldithio)oestra-1,3,5(10)-triene		Affinity labelling	446
Oestrogen receptor	Human-breast cancer cells	[^{3}H]Tamoxifen-aziridine		Selective labelling in whole cell system	447
Olfactory receptor	Rat olfactory mucosa	Mersalyl	Cys	Olfactory mechanism study	448
Opiate receptor	Rat brain	2-Nitro-4-azidophenyl derivatives of D-Ala-Leu-enkephalin		Photoaffinity labelling	449
δ-Opiate receptor	Rat brain	Tyr-D-Ala-Gly-*p*-N_3MePhe-Gly, Tyr-D-Thr-Gly-*p*-N_3 Phe-Leu-Thr		Photoaffinity labelling	450
Opioid receptor	Guinea-pig ileum, mouse vas deferens	α-Chlornaltrexamine		Irreversible agonist and antagonist	451
Opioid receptor	Rat brain	2,2′-Dithiobis-(5-nitropyridine), DTNB, 5,5′-dithiobis-(2-nitro-*N*-2′-hydroxyethylbenzamide)	Cys	Agonist binding inhibited	452
Ornithine aminotransferase	Rat	DL-4-Amino[2,3-^{14}C]hex-5-ynoic acid		Probe of intracellular protein turnover	453
Ornithine decarboxylase	Rat liver	α-(Fluoromethyl)dehydroornithine and -putrescine		New irreversible inhibitors	454
Ornithine decarboxylase	Rat	(2*R*,5*R*)-6-Heptyne-2,5-diamine		More potent than α-difluoromethylornithine	455

Ovalbumin		Dimethylmaleic, citraconic, and maleic anhydrides		Effect of temperature and pH on reversal	456
Parvalbumin	Carp	2-[(Trifluoroacetoxy)mercuri]-4-fluorophenol, 4-(acetoxymercuri)phenyl azide		New SH-specific cross-linking agents	457
Penicillin-binding proteins	*E. coli*	Photoreactive β-lactam antibiotics		Characterization	458
Pepsin	Porcine	1,2-Epoxy-3-(4′-azido-2′-nitrophenoxy)propane, *p*-azidophenacyl bromide, *p*-azido-α-diazoacetophenone	$-CO_2H$ and others	Photoactivatable covalent labels	459
Peptide transporter	*Candida albicans*	4-Azidobenzoyl trimethionine		90% efficient blockade of transport	460

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Peptidyl transferase	*S. cerevisiae* ribosome	*p*-Nitrophenylcarbamylamino-acyl-tRNA		Analogue with 3′-penta-nucleotide does not label	461
Peroxidase	Horseradish	*o*-Dianisidine/EDC	$-CO_2H$	Spectrophotometric quantitation	462
Phenylalanyl-tRNA synthetase	*E. coli*	Adenosine 5′-trimetaphosphate		Both subunits modified	463
Phenylalanyl-tRNA synthetase	*E. coli*	*N*-Bromoacetyl-[^{14}C]-phenylalanyl-tRNA		Affinity-labelling study of co-operative effects	464
Phenylalanyl-tRNA synthetase	*E. coli*	*N*-Bromoacetyl phenylalanyl-adenylate		Both subunits labelled	465
Phenylalanyl-tRNA synthetase	*E. coli*	Various ethenoadenosine derivatives		Trimetaphosphate derivative of ϵ-ATP inactivates	466
Phenylalanyl-tRNA synthetase	*S. cerevisiae*	*p*-Hydroxymercuribenzoic acid	Cys	Study of substrate binding	467
Phenylethanolamine-*N*-methyl transferase	Bovine adrenal medulla	*S*-Adenosyl-L-methionine, $h\nu$		Inactivation, crosslinking	468
Phosphate translocator protein	Rat-liver mitochondrion	*N*-[3-(2,2,5,5-Tetramethyl-1-pyrrolidinyloxyl)] maleimide	Cys	Characterization by spin-labelling	469
Phosphatidylcholine transfer protein	Bovine liver	Diazirinophenoxy derivatives of phosphatidylcholine	Tyr-54	Location of lipid-binding site	470
Phosphocarrier protein III	*Salmonella typhimurium*	FITC	Lys	Binding to protein HPr	471
Phosphoenol pyruvate carboxylase	Maize leaves	DEP	His	Inactivation	472
6-Phosphogluconate dehydrogenase	*Candida utilis*	*N*-Pyrene maleimide	Cys, Lys	Intramolecular aminolysis links Lys to Cys	473
Phosphoglucose isomerase	Rabbit muscle	Phenylglyoxal, butane-2,3-dione	Arg	Different specificities	474

Phosphoglycerate kinase	Porcine muscle	DNTB	Cys	Kinetics: effects of substrates	475
Phosphoglycerate mutase	Baker's yeast	Glutaraldehyde	$-NH_2$	Kinetics of subunit reassociation	476
Phospholipase A_2	*Naja naja atra, naja naja nigricollis*	Carbodi-imide, semicarbazide	$-CO_2H$	Separation of activities	477
Phospholipase A_2	*Naja melanoleuca*	4-Chloro-3,5-dinitrobenzoate	Lys-6	Affects activity indirectly	478
Phosphotransacetylase	*Clostridium kluyveri*	*S*-Dimethylarsino-Co A	Cys	Inactivation by transesterification	479
Phosphorylase *b*	Rabbit skeletal muscle	FITC		Probe for cholate interaction	480

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Photosystem II protein(s)	*Pisum sativum*	Phenylglyoxal, cyclohexane-1,2-dione	Arg	Atrazine-binding abolished	481
Phytohaemagglutinin	*Phaseolus vulgaris*	6-*N*-(4-Azidobenzoyl)amino-hexanoic acid succinimidyl ester, 6-mercapto-5-(4-azidophenyl)hexanoic acid succinimidyl ester		Retention of lymphocyte-stimulating activity	482
Plasma proteins	Human, rat	D-3-Mercapto-2-methyl-propanoyl-L-proline	Cys	Study of acute renal failure	483
Plasminogen	Human plasma	Various reagents	Arg-32, Tyr-40, Trp-71, Lys-35	N.M.R. studies	484
Platelet glycoprotein Ib	Human	*N*-Succinimidyl-(4-azido-phenyldithio) propionate, 3,3′-dithiobis(sulpho-succinimidvl) propionate		Involved in thrombin binding	485
Prekallikrein	Chicken plasma	4-Methylumbelliferyl *p*-guanidinobenzoate		Fluorescent active-site titrant	486
Progesterone receptor	Human uterus	11α- and 16α-(bromoacetoxy)-progesterone	His, Met	Affinity labelling	487
Progesterone receptor	Chick oviduct	2′,3′-Dialdehyde ATP		Affinity label	488
Progesterone receptor	Chicken oviduct	Mercurials, alkylating agents, DTNB	Cys	Effects on hormone- and DNA-binding	489
Progesterone receptor	Chick oviduct	[17α-Methyl-^{3}H]-17α,21-dimethyl-19-norpregn-4,9-diene-3,20-dione		Photoaffinity labelling	490
Progesterone receptor	Chick	[17α-Methyl-^{3}H]-17,21-dimethyl-19-norpregn-4,9-dienc-3,20-dione		Two subunits labelled at 5% efficiency	491

Prolipoprotein	*E. coli*	[^{3}H]FDNB	Lys	Membrane-association study	492
Prostaglandin synthetase	Sheep seminal vesicles	[Acetyl-^{3}H]aspirin	Ser	Single Ser: inhibition	493
Protein kinase	*Torpedo californica*	8-Azido-[α-^{32}P]ATP		Association with acetylcholine	494
Protein kinase (cAMP-dependent)	Rabbit muscle	Dimethyl suberimidate	Lys	Regulatory and catalytic subunits crosslinked	495
Protein kinase II, c AMP-dependent	Porcine heart	Iodoacetic acid	Cys	Selective protection by aggregation	496
Protein kinase, cGMP-dependent	Bovine lung	8-Azido-[^{32}P]cAMP		Photoaffinity labelling	497
Proteins L6, L11, and [$(L12)_4 \cdot L10$]	*E. coli* MRE 600 ribosomes	IAEDANS, 5-iodoacetimido-fluorescein	Cys	Distance measurement by energy transfer	498

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Proteins S17 and L6	*E. coli*	5-Iodoacetamidofluorescein	Cys-86 (L6) Cys-58, 63 (S17)	Immunoelectron microscopy	499
Prothrombin	Bovine	Dithiobis-(succinimidyl) propionate	(Lys)	No Ca^{2+}-dependent dimerization	500
Purple-membrane proteins	*Halobacterium halobium*	L-(+)Tartaric acid dihydrazide/EDC	$-CO_2H$	Cleavable crosslinking	501
Pyrophosphatase	*Rhodospirillum rubrum* chromatophores	NEM, PCMB	Cys	Membrane potential suppressed	502
Pyruvate decarboxylase	Yeast	(*E*)-4-(4-Chlorophenyl)-2-oxo-3-butenoic acid		Suicide inhibitor	503
Pyruvate dehydrogenase	Porcine heart	Pyruvate		Paracatalytic inactivation	504
Pyruvate, phosphate dikinase	*Bacteroides symbiosus*	Pyridoxal 5′-phosphate	Lys	Essential Lys	505
Reaction-centre protein	*Rhodospirillum rubrum*	DCCI	$-CO_2H$	Highly specific for subunit M	506
Reaction centres	*Rhodopseudomonas sphaeroides*	Dithiobis(propionimidate), dimethyl suberimidate	Lys	Cytochrome *c* binding site located	507
Reverse transcriptase	Avian myeloblastosis virus	ATP 2′,3′-dialdehyde		Essential thiol	508
Reverse transcriptase	Avian myeloblastosis virus	Rose Bengal, $h\nu$		Inactivation	509
Rhodopsin	Bovine retina	16-[^{15}N]Doxylstearic acid, *N*-(2-hydroxyethyl) maleimide ester	Cys	Lipid-interaction study	510
Ribonucleotide reductase	*Corynebacterium nephridii*	CPP and ADP, 2′,3′-dialdehyde derivatives		Affinity labelling displaces PPi	511
Ribonucleotide reductase	*E. coli*	2′-Azido-2′-deoxynucleoside diphosphates		Free-radical intermediates	512
Ribunucleotide reductase	*E. coli*	dTTP, dATP		Direct photoaffinity labelling	513

Ribonucleotide reductase	*Lactobacillus leichmannii*	3′-[^{3}H]-2′-Chloro-2′-deoxyuridine 5′-triphosphate		Inactivation releases ^3H_2O and eliminates Cl^-	514
Ribonucleotide reductase	Mouse	Cytosine [^{35}P]diphosphate		Direct photolabelling of MI subunit	515
Ribosomal protein	*E. coli*	2-Iminothiolane, O_2	$-NH_2$	50S ribosome structure	516
Ribosomal proteins	*E. coli*	Chlorambucilyl-$(Pro)_n$-Phe-$tRNA^{Phe}$		Crosslinking to 50S subunit	517
Ribosomal proteins	*E. coli*	2-Iminothiolane, *hν*		30S–RNA crosslinks	518
Ribosomal proteins	*E. coli*	2-Iminothiolane, O_2	Cys	Crosslinks identified	519

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Ribosomal proteins (30S)	*E. coli*	$Na^{125}I$, lactoperoxidase	Tyr	Topography study	520
Ribosomal proteins (30S)	*E. coli*	Pyridoxal phosphate/$NaBH_4$	Lys	Aminoacyl-tRNA and -mRNA binding sites modified	521
Ribosomal proteins (50S)	*E. coli*	Pyridoxal phosphate/$NaBH_4$	Lys	Inactivation of elongation-G-dependent GTPase	522
Ribosomal proteins (50S, 70S)	*E. coli*	$Na^{125}I$, lactoperoxidase	Tyr	Tyr identified	523
Ribosomal protein (70S)	*E. coli* MRE 600	6′-*N*-*p*-[^{3}H]Azidobenzyl tobramycin		Site of labelling investigated	524
Ribosomal protein L7/L12	*E. coli*	Mercaptopropionyl hydrazide, 3-(phenyldithio) propionimidate	Ser, Thr Lys	Introduction of —SH into proteins	525
Ribosomal protein S12	*E. coli*	4-(2′,4′-Dinitrophenylamino)-1-iodoacetylaminobutane	Cys	Immunological surface probe	526
Ribosomes	*E. coli*	Ethylene glycol bis[3-(2-ketobutyraldehyde) ether]	Arg	New RNA–protein cross-linker	527
Ribosomes (70S)	*E. coli*	Phenyldiglyoxal	Lys, Arg	RNA–protein contacts identified	528
Ribulose 1,5-bisphosphate carboxylase	Spinach	Tetranitromethane	Cys	Inactivation	529
Ribulose bisphosphate carboxylase/oxygenase	*Rhodospirillum rubrum*	2-[(Bromoacetyl)amino]pentitol 1,5-bisphosphate		Active-site peptide identified	530
Ricin	*Ricinus communis*	4-Azidophenyl- and 4-diazophenyl-β-D-galactopyranoside		Photoaffinity labelling	531
Ricin A-chain/immunoglobulin G	*Ricinus communis*/rat	SPDP		Association with ricin B-chain	532
Ricin B-subunit/insulin	*Ricinus communis*/porcine	SPDP	Lys	Insulin activity mediated *via* ricin receptor	533

RNA polymerase	*E. coli*	5′-{[(4-Azidophenacyl)thiol]-phosphoryl}adenylyl(3′-5′)-uridine		Cleavable photoaffinity probe	534, 535
RNA polymerase	*E. coli*	Phenylglyoxal, NEM, carbodi-imides	Arg, Cys, $-CO_2H$	Essential carboxyl	536
RNA polymerase II	HeLa cells	MeHgCl, *p*-hydroxymercuri-benzoate, NEM	Cys	Mechanism of Hg stimulation	537
RNase	Glucose		Lys	*Via* Schiff base	538
RNase	Bovine	Maleic anhydride iron tetracarbonyl	Lys	Enzyme inactivated	539

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
RNase A	Bovine pancreas	D-[6-^{3}H]Glucose	Lys	Study of non-enzymatic glucosylation and crosslinking	540
RNase A		Glycolaldehyde	$-NH_2$	Amadori rearrangement	541
RNase M	*Aspergillus saitoi*	CMC	$-CO_2H$	Inactivation	542
Sarcosine oxidase	*Corynebacterium*	(i) PCMB, DTNB, iodo-acetamide	(i) Cys	(i) Two essential Cys	543
		(ii) DEP	(ii) His	(ii) One essential His	
Serine hydroxymethyl-transferase	Rabbit-liver mitochondrion	Various reagents	Cys	Thiol-reactivity study	544
Serine peptidases	Various	1-(*N*-CBz-aminoethyl)isopropyl phosphonofluoridate, 1-(*N*-CBz-amino-2-phenylethyl)isopropyl phosphonofluoridate	Ser	Amino acid analogues of DFP	545
Serine proteases	Various	3- and 5-benzyl-6-chloro-2-pyrone, 3- and 5-methyl-6-chloro-2-pyrone	Ser	Suicide inhibitors	546
Somatotropin dimer	Bovine	Dimethyl suberimidate, 3,3′-dithiobispropionimidate	(Lys)	Growth-promoting activity reduced	547
Spermidine/spermine *N′*-acetyltransferase	Rat liver	Butane-2,3-dione, phenylglyoxal, camphorquinone 10-sulphonic acid	Arg	Arg binds acetyl-CoA	548
Subtilisins	*B. subtilis*	Glyoxal	Arg	Two to four essential Arg	549
Succinate cytochrome *c* reductase		2-Azido-3-methoxy-6-methyl-5-geranyl-1,4-benzoquinone and analogues		Photoaffinity analogues of coenzyme Q	550
Succinyl-CoA synthetase	*E. coli*	ADP 2′,3′-dialdehyde		1 mol mol^{-1} $\alpha\beta$	551
Succinyl-CoA synthetase	*E. coli*	5′-*p*-Fluorosulphonylbenzoyl-adenosine	Cys	Disulphide bond formation *via* thiosulphonate	552

Sucrase isomaltase	Rabbit-brush border membrane	3-{Dimethyl-2-([^{3}H]acetimidoxy-ethyl)ammonio}propane sulphonate	α-NH_2	New dipolar ionic reagent	553
Superoxide dismutase	*Pseudomonas ovalis*	Glutaraldehyde	(—NH_2)	Crystal stabilization for *X*-ray diffraction	554
Superoxide dismutase (Cu^{2+}, Zn^{2+})	Bovine	HO_2^-	His	True affinity inactivation	555
Superoxide dismutase (Cu^{2+}, Zn^{2+})	*S. cerevisiae*	HO_2^-	His	One His per subunit modified	556
Superoxide dismutase (Cu^{2+}, Zn^{2+})	Bovine	Potassium cyanate, succinic anhydride	Lys	Anion affinity, not metal co-ordination, affected	557
Terminal deoxy-nucleotidyltransferase	Calf thymus	8-Azido-ATP		Photoaffinity labelling	558

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Tetanus toxoid	Various	Glutaraldehyde, various peptides		Polyvalent synthetic vaccines	559
Thermolysin	*Bacillus stearothermophilus*	NBS	Trp	Activity unaffected (*cf.* talopeptin)	560
Thermolysin	*B. thermoproteolyticus*	$ClCH_2CO$-DL-(*N*-OH)Leu-OMe	Glu	Crystal structure	561
Thiamine pyrophospho-kinase	Rat liver	DTNB	Cys	Inhibition, Mg^{2+} protects	562
α-Thrombin	Human plasma	Tosyl-Lys-CH_2Cl, DFP	Ser, His	Chemotaxis independent of active site	563
Thrombin-binding sites	Chinese-hamster lung cells	Various nitroarylazide conjugates of α-thrombin		150K cell component labelled	564
Thymidylate synthetase	*Lactobacillus casei*	5-*p*-Benzoquinonyl-2′-deoxyuridine 5′-phosphate	Cys	Possible mechanism-based inhibitor	565
Thyroglobulin	Human thyroid	Succinic anhydride	$(-NH_2)$	Succinylation releases 26K polypeptide	566
T-Cell growth factor, TCGF receptor	JURKAT leukaemic cells, HUT-102B2 cells	Disuccinimidyl suberate			567
Toxin α	*Naja nigricollis*	(i) HCOOH/HCl, 2-nitrophenyl sulphenyl chloride (ii) [^{14}C]Acetic anhydride	(i) Trp (ii) Lys	Binding of non-modified toxins to receptor	568
α-Toxin	*S. aureus:* Wood 46	12-(4-Azido-2-nitrophenoxy)-stearoyl-1-^{14}C		Photolabelling from within cell membrane	569
Toxin V	*Leiurus quinquestriatus quinquestriatus*	Various fluorescent reagents	$-NH_2$	Study of receptor site	570, 571
Toxin receptors	Scorpion	4-Azido-2-nitrophenylglycyl α and β scorpion toxins		253K and 35K peptides labelled	572
Transferrin	Human, rabbit	Iodogen/Na^{125}I	Tyr	99.5% labelling as mono- and di-iodo-Tyr	573

Transferrin	Human serum, human milk, and hen egg-white	IO_4^-, H_2O_2	Tyr, Met	Essential Tyr	574
Transketolase	Yeast	Butane-2,3-dione	Arg	Essential Arg	575
Triose phosphate isomerase	Chicken muscle	Ferrate	Trp, His	Rapid inactivation	576
Tropomyosin	Rabbit heart	Bis-dansylcystine	Cys-190	Two conformational states found	577
Troponin C	Rabbit muscle	Dansylaziridine, IAEDANS	Met-25, Cys-98	Study of Ca^{2+} binding	578
				Study of subunit structure	579
Trp aporepressor	*E. coli* W 3110	Glutaraldehyde, dimethyl suberimidate	($-NH_2$)		

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Table *(cont.)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Trypsin	Bovine	Linoleic acid mixed anhydride	$-NH_2$	Immobilization on liposomes	580
Trypsin	*Streptomyces erythraeus*	PMSF	Ser	N.m.r. titration study	581
Trypsin	Bovine	*trans*-Cinnamoyl imidazole/$h\nu$	Ser	Autocatalytic enzyme amplification of light signals	582
Trypsin-like enzymes	Various	Benzyl *p*-guanidinothiobenzoate	Ser	Thiol release allows titration	583
Trypsin-like enzyme protease	Walker 256 carcinosarcoma	Various tripeptide chloromethyl ketones		Membrane-bound enzyme	584
Tryptophan synthase	*E. coli*	Various fluorescent reagents	Cys	4^y structure study	585
Tryptophan synthase α-subunit	*E. coli*	5-Azidoindole		Photoaffinity labelling	586
Tubulin	Lamb brain	3′-*p*-Azidobenzoyl-GTP, 2-(guanylformylmethoxy)-3-(triphospho)propanal		Affinity labelling	587
Tubulin dimer	Bovine brain	$HCHO/NaCNBH_3$	Lys	Reductive methylation blocks assembly	588
Tyrosinase	*Agaricus bisporus*	$h\nu$, citrate	His	Inactivation	589
Tyrosyl kinase, MT Ag-associated	Polyoma virus	NEM	Cys	Essential Cys	590
UDP-glucose-4-epimerase	*Saccharomyces fragilis*	PCMB	Cys	Subunit dissociation	591
UDP-glucuronyl transferase	Porcine liver	Butane-2,3-dione	Arg	Arg in active site	592
Urease	Jack bean	DTNB	Cys	Single essential Cys	593
Urease	Jack bean	Methylene Blue, $h\nu$	His	Inactivation	594
Various		6-Acryloyl-2-dimethyl-aminonaphthalene	Cys	Polarity-sensitive fluorescent probe	595

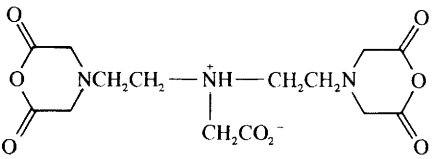
Various	Goat, rabbit	Biotin-*N*-hydroxysuccinimide, SPDP		Antibody-directed target of vesicles to cells	596
Various	Various	[3,5-^{3}H]FDNB	N-Terminal —NH,	Double-isotope method for identifying N-terminal	597
Vasopressin receptor	Bovine kidney	[1,6-α-Aminosuberic,3-(*p*-azidophenylalanine),8-arginine]-vasopressin		Photoaffinity label	598

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2 Investigations of Known and Novel Reagents and Reactions

Tetranitromethane. – An example of the modification of cysteine by this reagent, possibly *via* the sulphenyl nitrate, has been reported.[599] More than a third of those thiols in spinach ribulose 1,5-bisphosphate carboxylase titratable by Ellman's reagent were lost upon complete inactivation, and about half of these were restored by dithiothreitol, about a third of the modified thiol groups being irreversibly oxidized. Little or no tyrosine modification occurred (unlike the *Rhodospirillum rubrum* enzyme), although this finding is at odds with previous work on the spinach enzyme. No irreversible crosslinking was detected.

Diethylenetriamine Penta-acetic Anhydride. – As an improvement on methods for labelling IgG for radioimmunological tumour detection, a means of incorporating ^{111}In (which has better imaging characteristics than ^{131}I) has been developed.[600] The protein was reacted with the anhydride of the strong chelator diethylenetriamine penta-acetic acid (1), followed by addition of ^{111}In. No protein crosslinking was seen under conditions that gave 0.7 DTPA groups per molecule of IgG.



(1)

5,5′-Dithiobis-(2-nitrobenzoic Acid). – A review of this reagent[601] has re-assessed conditions for its use with proteins, in the light of recent findings concerning its chemical and spectral properties. A revised procedure for determining the thiol content of a protein is described.

Di-isopropyl Phosphorofluoridate. – In a study of the ageing of DFP-labelled neurotoxic esterase, Williams[602] has shown that the intramolecular isopropyl-group transfer responsible for the phenomenon is independent of the tissue source and that DIP-neurotoxic esterase has a distinctive and characteristic mode of ageing.

Other Reagents. – Two new reagents for introducing thiol groups into proteins have appeared:[603] mercaptopropionyl hydrazide specifically modifies periodate-

[599] G. Barnard, H. Roy, and Y. P. Myer, *Biochemistry*, 1983, **22**, 2697.
[600] P. J. Hnatowich, W. W. Layne, R. L. Childs, D. Lanteigne, M. A. Davis, T. W. Griffen, and P. W. Doherty, *Science*, 1983, **220**, 613.
[601] P. W. Riddles, R. L. Blakely, and B. Zerner, *Methods Enzymol.*, 1983, **91**, 49.
[602] D. G. Williams, *Biochem. J.*, 1983, **209**, 817.
[603] J. A. Maassen, T. P. G. M. Thielen, and W. Möller, *Eur. J. Biochem.*, 1983, **134**, 327.
[604] R. Bürgi, J. Brunner, and G. Semenza, *J. Biol. Chem.*, 1983, **258**, 15 114.

oxidized N-terminal Ser or Thr, and 3-(phenyldithio)propionimidate introduces a disulphide bond at lysine, retaining the charge and allowing reduction to a thiol. The latter compound reacts with high specificity at only one Lys residue of ribosomal protein L7/L12, compared with the more random modification found with iminothiolane.

A new dipolar imidoester, 3-(dimethyl-2-[^{3}H]acetimidoxyethylammonio)-propane sulphonate, has been reported, and it has been used to investigate the orientation of the small intestinal membrane enzyme sucrase-isomaltase.[604] With high reactivity at or near pH 7, the reagent has low permeability to brush-border membranes, although proteins with blocked N-termini will not be amenable to this type of analysis.

Rate constants have been determined for the deacylation of products of the reaction of ovalbumin with citraconic, maleic, and dimethylmaleic anhydrides over a range of conditions (0–40 °C, pH 4–8).[605]

The ferrate ion, FeO_4^{2-}, a strong oxidizing agent, is site-specific for protein phosphoryl sites by virtue of its physico-chemical and steric resemblance to phosphate. For triose phosphate isomerase its target residues have been identified as Trp-168, Trp-191, Tyr-164, and His-248.[606]

A highly specific modification of His residues (one out of sixteen) in a glutamine synthetase/ascorbate model system may involve Fe^{2+} and the formation of an endoperoxide.[607] *In vivo* this type of oxidative modification could serve to label the protein for subsequent metabolic proteolysis.

Phosphorus oxychloride has been shown to improve the emulsifying properties of β-lactoglobulin by converting amino groups to phosphoramidates at a level of 13 mol P mol^{-1} protein monomer.[608]

Reviews of the following modifications have been published: protein amino-terminal analysis with dimethylaminoazobenzene isothiocyanate,[609, 610] amidination,[611] and labelling by reductive alkylation.[612]

3 Modifications for Physical Techniques

Two publications have illustrated the potential of metallocene complexes for introducing metal ions into proteins as spectroscopic probes. In the first,[613] tetramethylcyclobutadiene nickel dichloride gave an orange, soluble adduct with horse-liver dehydrogenase. Eight atoms of Ni were incorporated per mole of protein, probably *via* Cys residues, and the adduct showed a positive Cotton

[605] M. A. Nieto and E. Palacian, *Biochim. Biophys. Acta*, 1983, **749**, 204.
[606] J. Steczko, M. Hermodson, B. Axelrod, and E. Dziernbor-Kentzer, *J. Biol. Chem.*, 1983, **258**, 13 148.
[607] R. L. Levine, *J. Biol. Chem.*, 1983, **258**, 11 828.
[608] S. L. Woo and T. Richardson, *J. Dairy Sci.*, 1983, **66**, 984.
[609] J. Y. Chang, *Methods Enzymol.*, 1983, **91**, 79.
[610] J. Y. Chang, *Methods Enzymol.*, 1983, **91**, 455.
[611] J. K. Inman, R. N. Perham, G. C. DuBois, and E. Appella, *Methods Enzymol.*, 1983, **91**, 559.
[612] N. Jentoft and D. G. Dearborn, *Methods Enzymol.*, 1983, **91**, 570.
[613] B. Foucaud and J. F. Biellmann, *Biochim. Biophys. Acta*, 1983, **748**, 362.

effect. In the second report,[614] maleic anhydride–$Fe(CO)_4$ reacted with RNase at Lys-41, giving 1 atom Fe mol^{-1} enzyme. $Fe(CO)_4$ has visible absorption for optical density and optical rotation measurements, a carbonyl bond for infrared spectroscopy, and metal-electron density for crystallography.

Another reagent with crystallographic applications is methylphenyl mercury.[615] In carboxyhaemoglobin it reacted with two Cys residues that are normally inaccessible (G11 104α and G14 112β), but not with the more usual exposed Cys F9 93β. It may therefore be used for labelling hydrophobic protein interiors. Such a distinction between exposed and buried Cys residues has also been made in the mitochondrial phosphate translocator,[616] using *N*-[3-(2,2,5,5-tetramethyl-1-pyrrolidinyloxyl)]-maleimide.

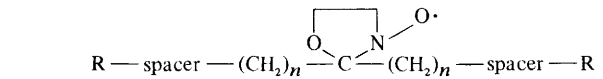
The value of chemical modifications in studying protein structure by n.m.r. has been exemplified by work on kringle 4 of human plasminogen,[617] where various reactions at specific positions (residues modified include Arg, Trp, Tyr, Cys, and Lys) have been used for assigning n.m.r. resonances to define the solution structure of the kringle fold. Also, in horse cytochrome *c*, Lys residues have been modified by various reagents[618] in order to investigate the effects of Tyr and Lys ionizations on protein conformation by examining temperature and pH changes in the spectra. ^{19}F n.m.r. has been used[619] to determine rate-constant and activation parameters for restricted rotation about the C—N bond in bis-(4-fluorophenyl)carbamoyl α-chymotrypsin. Stoicheiometric carbamoylation gave rise to two broad signals 3.4 p.p.m. apart.

Spin-labelling has continued to be a fruitful means of probing protein structure: for example, by labelling a single thiol in aconitase with maleimide and bromoacyl spin-labels, the distance between this residue and the Fe—S cluster has been calculated.[620] In aspartate aminotransferase, a spin-labelled oxaloacetate analogue, potassium nitrosodisulphonate, has been developed as a probe[621] for the active-site Trp-140. Gabibov *et al.*[622] have combined e.s.r. (using 2,2,5,5-tetramethyl-3-dichlorotriazinylaminopyrrolidin-1-oxyl, introduced at Tyr-72 by nitration/reduction) and stopped-flow measurements to investigate the interaction between cAMP-dependent histone kinase and histone H1, for which an association constant was calculated. Girardet and Lawrence[623] used 5-fluoro-2,4-dinitroanilino and imidazole spin-labels to modify, respectively, Lys

[614] R. W. Giese, *J. Inorg. Biochem.*, 1983, **18**, 301.
[615] D. J. Abraham, S. E. V. Phillips, and P. E. Kennedy, *J. Mol. Biol.*, 1983, **170**, 249.
[616] J. Houstek, E. Bertoli, I. Stipani, S. Pavelka, F. M. Megli, and F. Palmieri, *FEBS Lett.*, 1983, **154**, 185.
[617] M. Trexler, L. Bányai, L. Patthy, N. D. Pluck, and R. J. P. Williams, *FEBS Lett.*, 1983, **154**, 311.
[618] A. P. Boswell, G. R. Moore, R. J. P. Williams, D. E. Harris, C. J. A. Wallace, S. Bocieck, and D. Weltz, *Biochem. J.*, 1983, **213**, 679.
[619] M. Cairi and J. T. Gerig, *J. Am. Chem. Soc.*, 1983, **105**, 4793.
[620] J.-L. Dreyer, H. Beinhert, J. F. W. Keana, O. H. Hankovszky, K. Hideg, S. S. Eaton, and G. R. Eaton, *Biochem. Biophys. Acta*, 1983, **745**, 229.
[621] A. Iriarte and M. Matrinez-Carrion, *J. Biol. Chem.*, 1983, **258**, 2217.
[622] A. G. Gabibov, S. N. Kochetkov, L. P. Sashchenko, I. V. Smirnov, V. P. Timofeev, and E. S. Severin, *Eur. J. Biochem.*, 1983, **132**, 339.
[623] J. L. Girardet and J. J. Lawrence, *Eur. J. Biochem.*, 1983, **132**, 195.

in the basic and N- and C-terminal regions and Tyr in the hydrophobic parts of histone H1, thus allowing a comparison of their respective roles in the interaction with nucleosomal DNA.

Willingham and Gaffney[624] have introduced a novel type of spin-label of general structure (2), in which the geometry of the 3-oxyoxazolidine ring is such that the hyperfine splitting in the e.s.r. spectrum is optimized for studying the orientation of the reagents within a membrane. Their reagents have chains sufficiently long to span a membrane, and polar or reactive groups at each end. The exact structure of the reagents determines whether the molecule will span the membrane or bind with both functionalities at the surface. Two examples were selected for intensive study with erythrocytes: 3-oxy-2,2-bis-{6-[(*N*-succinimidyloxy)carbonyl]}-4,4-dimethyl oxazolidine passed through intact erythrocyte membranes and reacted almost exclusively with Hb, allowing study of the protein's rotational mobility within the red cell, and 3-oxy-2,2-bis-{[({2-[(3-carboxy-4-nitrophenyl)[^{35}S]dithio]ethyl}amino)carbonyl]hexanyl}-4,4-dimethyl oxazolidine, which is cleavable to allow analysis of crosslinked proteins, reacted with membrane components as well as with Hb.

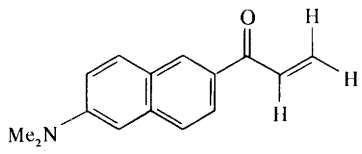


(2)

Other reported uses of spin-labels include the detection by *N*-(1-oxy-2,2,6,6-tetramethyl-4-piperidinyl) iodoacetamide of two Ca^{2+}-induced conformational states in sarcoplasmic reticulum ATPase[625] and the use of 4-amino-2,2,6,6-tetramethyl piperidine-*N*-oxyl to locate a buried carboxyl in a bacteriorhodopsin hydrophobic domain >16 Å from the membrane surface.[626] Lastly, by using phenyl-*N*-t-butyl nitrone to spin-trap a sulphur radical generated from albumin or myosin in the presence of Ce^{4+}, a strongly immobilized nitroxide spin-label has been attached to a protein much closer to the polypeptide backbone than would be possible with conventional reagents.[627]

Two novel fluorescent reagents have been reported this year. The first is a Koshland reagent, 2-carboxyl-1-hydroxyl-4-naphthylmethyldimethyl sulphonium chloride,[628] which reacted rapidly with Trp under mild conditions; in acidic solutions the only other weakly reactive residue was Cys. The specificity was low in alkaline media. The second new reagent,[629] 6-acryloyl-2-dimethylaminonaphthalene [Acrylodan (3)], labelled Cys selectively, unlike other commercially available 6-acyl-2-dimethylaminonaphthalene derivatives, which often interact

[624] G. L. Willingham and B. J. Gaffney, *Biochemistry*, 1983, **22**, 892.
[625] C. Coan, *Biochemistry*, 1983, **22**, 5826.
[626] J. M. Herz, R. J. Mehlhorn, and L. Packer, *J. Biol. Chem.*, 1983, **258**, 9899.
[627] P. Graceffa, *Arch. Biochem. Biophys.*, 1983, **225**, 802.
[628] T. Hojo, H. Nakamura, Z. Tamura, and T. Nakajima, *Chem. Pharm. Bull.*, 1983, **31**, 3350.
[629] F. G. Prendergast, M. Meyer, G. L. Carlson, S. Iida, and J. D. Potter, *J. Biol. Chem.*, 1983, **258**, 7541.



(3)

non-covalently with proteins. In all proteins studied, Acrylodan was sequestered in hydrophobic pockets, and, because of the lack of flexible substituents, spectral shifts observed in different environments reflected true changes in polarity.

The potential of fluorescence measurements for determining intra- and intermolecular distances in proteins has been widely exploited, and Genov[630] has shown that the distances obtained (in this case the Trp-Ser distance in dansyl-subtilisin Novo) can agree very well with those from *X*-ray co-ordinates. In an investigation of the quaternary structure of *E. coli* Trp synthetase,[631] fluorescence energy-transfer distance measurements were used, together with sedimentation and diffusion parameters, to discriminate between a large number of possible subunit arrangements, and the final models agreed well with those derived from independent small-angle *X*-ray scattering and chemical crosslinking data. However, in a study of ribosome assembly using fluorescein attached to defined residues in component proteins,[632] it was shown that, while the use of a fluorophore-specific antibody allows distance determinations by fluorimetry and immunoelectron microscopy, the values obtained by the former technique are smaller. Both this study and another[633] (in which ribosomal proteins L6, L10, and L11 were labelled using IAEDANS or IAF) demonstrate that the attachment of labels to single, defined sites gives results that are numerically and topographically less ambiguous than those from random, multiple labelling.

An interesting discrimination between different protein environments has been reported for rabbit cardiac tropomyosin specifically labelled at Cys-190 with didansyl cystine.[634] The fluorescence decay comprised a major component of lifetime 6 ns and a minor blue-shifted component of lifetime 17 ns, due to sampling by the probe of polar and hydrophobic environments, respectively. The structure-unfolding transition gave the probe greater access to the hydrophobic region, which was reflected in an increase in the minor component. Gupte and Lane[635] used two reagents, 2-(4′-maleimidyl anilino)naphthalene-6-sulphonic acid and *N*-(1-pyrene)maleimide, to alkylate Cys residues in (Na,K)-ATPase, and they found different specificities for the two reagents, the bulkier *N*-(1-pyrene)-maleimide reacting with more hydrophobic environments.

[630] N. Ch. Genov, M. Shopova, R. Boteva, F. Rickhelli, and G. Joli, *Biochem. J.*, 1983, **215**, 413.
[631] A. N. Lane and K. Kirschner, *Eur. J. Biochem.*, 1983, **129**, 675.
[632] M. Stöffler-Meilicke, B. Epe, K. G. Steinhäuser, P. Woolley, and G. Stöffler, *FEBS Lett.*, 1983, **163**, 94.
[633] K. G. Steinhäuser, P. Woolley, J. Dijk, and B. Epe, *Eur. J. Biochem.*, 1983, **137**, 337.
[634] D. R. Betteridge and S. S. Lehrer, *J. Mol. Biol.*, 1983, **167**, 481.
[635] S. S. Gupte and L. K. Lane, *J. Biol. Chem.*, 1983, **258**, 5005.

Other proteins for which fluorescence measurements have been used to obtain distance information include myosin,[636,637] actin,[637] cytochrome C,[638,639] membrane-bound IgE,[640] and scorpion toxin V.[641,642]

4 Crosslinking

A review covering a wide range of crosslinking agents has appeared,[643] together with reviews on membrane crosslinking[644] and protein-conjugate synthesis.[645]

This year relatively few novel crosslinking agents have been described, indicating perhaps that the stock of published and commercially available agents is now able to cater for most requirements. There is a trend towards replacing succinimidyl esters with the more water-soluble sulphosuccinimidyl esters; a new heterobifunctional agent, *N*-succinimidyl (4-iodoacetyl)aminobenzoate, has also been prepared as the sulphosuccinimidyl analogue.[646] Bis-(sulphosuccinimidyl) suberate has been used to demonstrate a specific interaction between calmodulin and β-endorphin (in purified states).[647] One additional feature of sulphosuccinimidyl esters is that lysine selectivity may be affected by adjacent positively or negatively charged residues; this, however, might be a disadvantage when crosslinking preformed complexes.

Two CNBr-reversible crosslinkers, oxalyl- and malonyl-bis(methionyl-*p*-nitrophenyl esters), have been synthesized[648] for peptide-synthesis purposes: the A1 and B29 residues of insulin were crosslinked to ensure correct pairing of cysteines during oxidation. Unlike other reversible agents, the reversal regenerates unmodified peptide (or protein), but unfortunately few proteins are devoid of methionine.

Glycolaldehyde has been shown to function as a crosslinking agent;[649] the Schiff base formed can undergo an Amadori rearrangement to give an aldoamine. This is capable of reacting with a second protein amino group to give a yellow, fluorescent crosslink. The compound may be of use for situations where two amino groups lie close together (deoxyhaemoglobin S is suggested), but for intermolecular crosslinking good yields of crosslinked species will probably involve extensive lysine modification.

[636] R. E. Dalbey, J. Weiel, and R. G. Yount, *Biochemistry*, 1983, **22**, 4696.
[637] H. R. Trayer and I. P. Trayer, *Eur. J. Biochem.*, 1983, **135**, 47.
[638] W. Schwarze, R. Bernhardt, G.-R. Janig, and K. Ruckpaul, *Biochem. Biophys. Res. Commun.*, 1983, **113**, 353.
[639] R. Bernhardt, N. T. Ngoc Dao, H. Steil, W. Schwarze, J. Friedrick, G.-R. Janig, and K. Ruckpaul, *Biochim. Biophys. Acta*, 1983, **745**, 140.
[640] D. Holowka and B. Baird, *Biochemistry*, 1983, **22**, 3475.
[641] K. J. Angelides and T. J. Nutter, *J. Biol. Chem.*, 1983, **258**, 11 948.
[642] K. J. Angelides and T. J. Nutter, *J. Biol. Chem.*, 1983, **258**, 11 958.
[643] T. H. Ji, *Methods Enzymol.*, 1983, **91**, 580.
[644] C. R. Middaugh, E. F. Vanin, and T. H. Ji, *Mol. Cell. Biochem.*, 1983, **50**, 115.
[645] A. H. Blair and T. I. Ghose, *J. Immunol. Methods*, 1983, **59**, 129.
[646] J. K. Weltman, S. A. Johnson, J. Langeuin, and E. F. Riester, *Biotechniques*, 1983, **1**, 148.
[647] D. P. Gieroc, D. Puett, N. Ling, and J. V. Staros, *J. Biol. Chem.*, 1983, **258**, 16.
[648] B. R. Srinivasa and F. H. Carpenter, *Int. J. Pept. Protein Res.*, 1983, **22**, 214.
[649] A. Seetharama and J. M. Manning, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 3590.

A new α-dicarbonyl RNA–protein crosslinker, ethylene glycol bis-[3-(2-ketobutyraldehyde)ether], has been reported and used to study the *E. coli* ribosome.[650] The reagent, which can be prepared in one step, gives crosslinks that are reversible by removal of the stabilizing borate ions.

5 Affinity Labelling

Two papers have been published dealing with kinetic aspects of affinity labelling. The first[651] deals with situations where a biphasic loss of activity is seen and the transition point is independent of substrate concentration. The model developed is a two-states model; only a small fraction of the enzyme, in the order of 10% for $(Na^+ + K^+)$- and (Ca^{2+})-ATPase, exists in an active state, and this is inactivated rapidly. Conversion of the inactive state to the active state is relatively slow, resulting in the slow second phase of inactivation.

In the second paper, a model dealing with dimeric enzymes with identical ligand-binding sites is described.[652] Data on the inactivation of phenylalanyl-tRNA synthetase by *N*-bromoacetyl-[^{14}C]phenylalanyl-tRNA are treated in terms of this model, and strong negative co-operativity was demonstrated in this case.

Nucleotide-binding Sites. – The affinity labelling of purine nucleotide sites has been reviewed.[653] 2′,3′-Dialdehyde derivatives of adenine nucleotides have been used extensively, and this method has been extended to GTP for the study of tubulin.[654] 5′-(4-Fluorosulphonylbenzoyl)adenosine has been used to affinity-label oligomeric casein kinase,[655] rat-liver carbamyl phosphate synthetase,[656] and the *E. coli* succinyl-CoA synthetase.[657] In this latter case, inactivation was attributed to disulphide bond formation proceeding *via* a thiosulphonate intermediate formed as a result of attack of a reactive thiol on the reagent. The EGF receptor/protein kinase has also been affinity-labelled with the radiolabelled reagent.[658] By purification of the labelled receptor on immobilized EGF, it was shown that the receptor and the (EGF-stimulatable) protein kinase are two parts of the same chain.

Some of the drawbacks of 5′-(4-fluorosulphonylbenzoyl)adenosine, *viz.* poor water solubility, lack of characterization of the amino acid derivative formed, and high rate of hydrolysis, have been considered.[659] The authors suggest 5′-bromoacetamide-5′-deoxyadenosine as an alternative, and with this they have affinity-labelled 3α,20β-hydroxysteroid dehydrogenase. The reagent is more

[650] L. A. Brewer, S. Goelz, and H. F. Noller, *Biochemistry*, 1983, **22**, 4303, 4310.
[651] G. Fritzsch and H. Koepsall, *J. Theor. Biol.*, 1983, **102**, 469.
[652] I. I. Gorshkova and O. I. Lavrik, *Biochim. Biophys. Acta*, 1983, **746**, 202.
[653] R. F. Coleman, *Ann. Rev. Biochem.*, 1983, **52**, 67.
[654] R. B. Maccioni and N. W. Seeds, *Biochemistry*, 1983, **22**, 1572.
[655] J. Feige *et al.*, *Biochemistry*, 1983, **22**, 1452.
[656] S. G. Powers, G. W. Muller, and N. Kafka, *J. Biol. Chem.*, 1983, **258**, 7545.
[657] A. R. S. Prasad, J. Ybarra, and J. S. Nishimura, *Biochem. J.*, 1983, **215**, 513.
[658] S. A. Buhrow, S. Cohen, D. L. Garbers, and J. V. Staros, *J. Biol. Chem.*, 1983, **258**, 7824.
[659] B. R. Samant and F. Sweet, *J. Biol. Chem.*, 1983, **258**, 12 779.

soluble (15 mM), relatively stable, gives well characterized carboxymethylamino acids upon hydrolysis, and, in addition, its affinity relative to adenosine is essentially unchanged.

Receptors. – 4-(*N*-Maleimido)benzyltrimethylammonium ions have been used to affinity-label nicotinic acetylcholine receptors from the lobster axon[660] and the locust supraoesophageal ganglion.[661] In the latter case at least, cysteine is the modified residue. The *Torpedo californica* receptor has been labelled with [^{3}H]quinacrine mustard.[662]

The human uterine progesterone receptor has been affinity-labelled with 11α- and 16α-(bromo[2′-^{3}H]acetoxy)progesterone, and the modified amino acids have been characterized.[663] The 11α reagent labelled position 1 of a histidine ring, whilst the 16α reagent labelled position 3 and a methionine residue. The oestrogen receptor has been studied with a tritiated aziridine derivative of tamoxifen.[664] Glucocorticoid receptors from human leukaemic and malignant lymphoma cells[665] and from rat hepatoma cells[665] have been labelled with [^{3}H]dexamethasone 21-mesylate. Conditions have now been found[666] that give labelling efficiencies of *ca.* 90%.

Among other membrane proteins studied are the slow inward calcium channel,[667] which has been labelled with a tritiated isothiocyano analogue of the blockers nitrendipine and nifedipine (a M_r 45 000 subunit was labelled), and the erythrocyte anion-transport[668] system, which was studied with [^{14}C]-4-benzamido-4′-isothiocyanostilbene 2,2′-disulphonate. A M_r 19 000 membrane-spanning domain of the band-3 polypeptide was labelled.

Enzyme Active Sites. – Given the widespread use of phosphorylating agents such as di-isopropyl phosphorofluoridate for the study of serine proteases, it is odd that hitherto little attempt has been made to include a substrate-like feature to give increased specificity and reactivity. Now, however, derivatives of Z-alanyl and Z-phenylalanyl phosphonofluoridate have been synthesized for use with elastase and chymotrypsin, respectively.[669] Of the elastase inactivators, the isopropyl ester is the most useful, with a second-order rate constant of 7.7×10^4 $M^{-1}\,min^{-1}$; the second-order rate constant for the chymotrypsin reagent is 1.1×10^{-7} $M^{-1}\,min^{-1}$. Thus, these compounds are the most potent inactivators yet reported for these enzymes, and they may prove to be of considerable value

[660] H. G. Mautner, J. L. Cole, and M. A. Belew, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 61.
[661] M. T. Filbin, G. G. Lunt, and J. F. Donnellan, *Eur. J. Biochem.*, 1983, **132**, 151.
[662] R.-R. J. Kaldany and A. Karlin, *J. Biol. Chem.*, 1983, **258**, 6232.
[663] S. D. Holmes and R. G. Smith, *Biochemistry*, 1983, **22**, 1729.
[664] J. A. Katzenellenbogen, K. E. Carlson, D. F. Heiman, D. W. Robertson, L. L. Wei, and B. S. Katzenellenbogen, *J. Biol. Chem.*, 1983, **258**, 3487.
[665] C. M. Foster, H. J. Eisen, and C. D. Bloomfield, *Cancer Res.*, 1983, **43**, 5273.
[666] S. S. Simons, R. E. Schleenbaker, and H. J. Eisen, *J. Biol. Chem.*, 1983, **258**, 2229.
[667] J. C. Venter, C. M. Fraser, J. S. Schaber, C. Y. Jung, G. Bolger, and D. J. Triggle, *J. Biol. Chem.*, 1983, **258**, 9344.
[668] S. Kitagawa, J. Endo, R. Kubo, and F. Kametani, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 306.
[669] L. A. Lampen and P. A. Bartlett, *Biochem. Biophys. Res. Commun.*, 1983, **112**, 1085.

in a wide range of biochemical investigations. A trypsin analogue would also be welcome.

Bis-peptide cystamine compounds, *e.g.* bis(Gly-Phe) cystamine, have been used as affinity reagents for cathepsin B.[670] The immobilized reagent can be used for covalent affinity chromatography; elution of the enzyme is achieved with 10 mM cysteine. Another disulphide reagent, methoxycarbonyl-CoA disulphide, has been reported for labelling carnitine acetyltransferase.[671] An essential thiol is modified *ca.* ten times faster than other thiols, and reversal is achieved with dithiothreitol. Inactivation of phosphotransacetylase by the related agent *S*-dimethylarsino-CoA is, however, not reversible by dithiothreitol.[672] Transfer of the dimethylarsino group to the enzyme thiol is implicated. This reagent does not inactivate carnitine acetyltransferase and citrate synthase.

Confirmation that 17β-oestradiol dehydrogenase is the same as 20β-hydroxysteroid dehydrogenase has come from a study with 6β-[2′-^{14}C]bromoacetoxyprogesterone.[673] The pattern of alkylation (Cys > His > Lys) shows that both activities arise from the same active site. Reaction of the enzyme with 3-bromo-[2′-^{14}C]acetoxyoestrone and 12β-bromo[2′-^{14}C]acetoxy-4-oestrone-3,17-dione causes the same histidine to be labelled, despite the distance between the two bromine atoms.[674] A similar phenomenon occurs with 3-oxo-Δ^5-steroid isomerase;[675] reaction with spiro-17β-oxiranyl-Δ^4-androster-3-one and spiro-17β-oxiranyloestra-1,3,5(10),6,8-pentaene-3-ol causes Asp-38 to be modified, as does the A-ring photolabel 3-oxo-4-oestren-17β-yl acetate. In this case, it is proposed that 'backwards-binding' modes are possible with these labels (it is not clear which of the above reagents binds in the correct way) and that care in the interpretation of such experiments is in order. Presumably, this care should be extended to other labels based on substrates that possess some form of crude symmetry.

Bovine and yeast superoxide dismutases are inactivated by H_2O_2 at alkaline pH with the loss of one histidine per subunit.[676,677] It is proposed that the peroxide anion is behaving as a true affinity reagent.

It is encouraging when affinity-labelling results can be interpreted in terms of a known *X*-ray structure. *S*-2-Chloro-3-(imidazol-5-yl) propionate inactivates alcohol dehydrogenase whilst the *R* enantiomer does not. Computer model-building studies have shown[678] that, although both enantiomers can bind to give a reversible complex, the second irreversible step is only possible, for steric reasons, for the *S* enantiomer.

[670] B. Evans and E. Shaw, *J. Biol. Chem.*, 1983, **258**, 10 227.
[671] V. Venkataraghavan and D. J. Smith, *Arch. Biochem. Biophys.*, 1983, **220**, 193.
[672] E. F. Duhr, M. S. Owens, and R. E. Barden, *Biochim. Biophys. Acta*, 1983, **749**, 84.
[673] J. L. Thomas and R. C. Strickler, *J. Biol. Chem.*, 1983, **258**, 1587.
[674] G. L. Murdock, C. C. Chin, R. E. Offord, R. A. Bradshaw, and J. C. Warren, *J. Biol. Chem.*, 1983, **258**, 11 460.
[675] R. H. Kayser, P. L. Bounos, C. L. Bevins, and R. M. Polack, *J. Biol. Chem.*, 1983, **258**, 909.
[676] H. J. R. Fuchs and C. L. Borders, jun., *Biochem. Biophys. Res. Commun.*, 1983, **116**, 1107.
[677] D. M. Blech and C. L. Borders, *Arch. Biochem. Biophys.*, 1983, **224**, 579.
[678] K. H. Dahl, H. Eklund, and J. S. McKinley-McKee, *Biochem. J.*, 1983, **211**, 391.

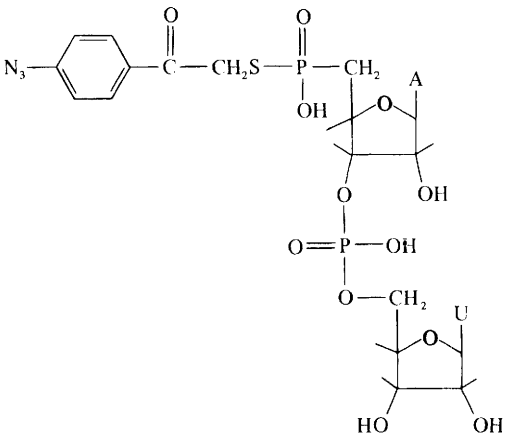
Finally, affinity labelling has been used to investigate the relationship between crystal and solution structures. Labelling of the aspartate aminotransferase isoenzymes with 4′-*N*-(2,4-dinitrofluorophenyl)pyridoxamine 5′-phosphate proceeds in the crystal as in solution,[679] *i.e.* the mitochondrial form cleaves to give pyridoxal 5′-phosphate, which reacts with the active-site lysine, whilst the cytoplasmic enzyme also proceeds *via* reaction of lysine with C-5 of the phenyl group.

6 Photoaffinity Labelling and Photocrosslinking

There can be little doubt that photoaffinity techniques are now of major importance in biochemistry and molecular pharmacology. This is reflected in the high proportion of papers listed in the Table that use photoactivation of ligands to label proteins specifically. It is also clear that the variety of chemical structures being photoactivated is steadily increasing and that mechanistic aspects of the labelling process present a continuing challenge. Aromatic and heteroaromatic azides are still the most widely used reagents, but the limitations placed upon design and interpretation of biological experiments by nitrene chemistry are becoming more apparent. For example, [^{125}I]iodonaphthyl azide has been shown to be unsuitable as a non-selective hydrophobic photolabel because the electrophilic nitrene derived from it labelled only a single cysteine residue in *E. coli* ATP synthase.[680] However, other 'hydrophobic' aryl azides have been used to probe membrane proteins, with apparent success.[681,682] Similarly, azido-substituted naphthalene sulphonates have proved to be useful photolabels for yeast cytochrome *c* oxidase because their photolysis products are fluorescent and can thus allow fluorographic detection of labelled peptides.[683] The nature of these fluorophores (possibly substituted benzazepines) is, however, still obscure. There have been several recent instances of azide-based photoaffinity labels being used successfully despite the possibility of efficient intramolecular rearrangement of the nitrene. For example, a 24-substituted digitoxoside containing an unsaturated azidomethyl ketone function has been used to photolabel porcine Na^+,K^+-activated ATPase;[684] in this case, labelling could have occurred *via* non-nitrene reactive intermediates such as an α-keto-imine. In addition, the presence of *ortho* substituents in azide-based photolabels is not necessarily inimical to protein labelling. A series of ubiquinone analogues *ortho*-substituted with azide and alkyl/alkoxy groups in the quinone ring have been prepared and reported to block electron-transfer processes upon irradiation.[685]

[679] S. Ottonello, A. Mozzarelli, G. L. Rossi, D. Carotti, and F. Riva, *Eur. J. Biochem.*, 1983, **133**, 47.
[680] J. Hoppe, P. Friedl, and B. B. Jorgensen, *FEBS Lett.*, 1983, **160**, 239.
[681] E. W. Steckel, B. E. Welbaum, and J. M. Sodetz, *J. Biol. Chem.*, 1983, **258**, 4318.
[682] K. B. Delclos, E. Yeh, and P. M. Blumberg, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 3054.
[683] M. E. Dockter and T. Koseki, *Biochemistry*, 1983, **22**, 3924.
[684] T. Deffo, D. S. Fullerton, M. Kihara, R. H. McParland, R. R. Becker, B. M. Simat, A. H. From, K. Ahmed, and M. I. Schimerik, *Biochemistry*, 1983, **22**, 6303.
[685] L.-Q. Gu, L. Yu, and C. A. Yu, *Biochem. Biophys. Res. Commun.*, 1983, **113**, 477.

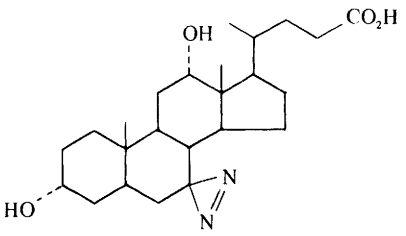
Azido derivatives of purine nucleotides have continued to be used very widely for photolabelling nucleotide-binding sites in proteins, and reviews of this topic have appeared.[686-688] A new cleavable dinucleotide label (4) derived from *p*-azidophenacyl bromide has been described and used to map the *E. coli* transcription complex;[689,690] the phosphorothioate linkage may be cleaved efficiently with organomercurials. Despite the popularity of azide analogues of nucleotide phosphates 'direct' photoaffinity labelling with the unmodified molecules themselves has also grown in importance. The majority of these experiments use short periods of irradiation with low-wavelength u.v. light (*e.g.* refs. 691, 692, and 693) and, although analogue synthesis can be avoided, these conditions of irradiation may be too harsh for many biological systems.



(4)

Diazirines have also continued to find new applications as photoaffinity labels, especially in membrane studies. ω-(*m*-Diazirino)phenoxyacyl phospholipid analogues[694,695] and 3-trifluoromethyl-3-*m*-[^{125}I]iodophenyl diazirine[696]

[686] E. Boyd Haley, *Fed. Proc.*, 1983, **42**, 2831.
[687] R. F. Colman, *Annu. Rev. Biochem.*, 1983, **52**, 67.
[688] R. L. Potter and B. E. Haley, *Methods Enzymol.*, 1983, **91**, 613.
[689] M. M. Hanna and C. F. Meares, *Biochemistry*, 1983, **22**, 3546.
[690] M. M. Hanna and C. F. Meares, *Proc. Natl. Acad. Sci. Soc. U.S.A.*, 1983, **80**, 4238.
[691] V. E. Groppi, F. Steinberg, H. R. Kaslow, N. Walker, and H. R. Bourne, *J. Biol. Chem.*, 1983, **258**, 9717.
[692] M. Berrios, G. Blobel, and P. A. Fisher, *J. Biol. Chem.*, 1983, **258**, 4548.
[693] I. W. Caras, T. Jones, S. Eriksson, and D. W. Martin, jun., *J. Biol. Chem.*, 1983, **258**, 3064.
[694] Y. Takagaki, R. Rahakrishnan, C. M. Gupta, and H. G. Khorana, *J. Biol. Chem.*, 1983, **258**, 9128.
[695] J. Westerman, K. W. A. Wirtz, T. Berkhout, L. L. M. Van Deenan, R. Radhakrishnan, and H. G. Khorana, *Eur. J. Biochem.*, 1983, **132**, 441.
[696] B. Y. Gysin and R. Schwyzer, *FEBS Lett.*, 1983, **158**, 12.



(5)

have proved particularly useful. An interesting example of photolabelling of bile-salt transporters with a diazirine precursor of an alicyclic carbene (5) has been reported by two groups.[697,698] Again, the apparent unimportance of intramolecular hydrogen shifts is perhaps surprising.

Photoactivated labels that are not necessarily strict photoaffinity reagents have included the triphenyl methyl phosphonium ion for acetylcholine receptor[699] and bleomycin for dopamine β-hydroxylase.[700] In neurochemical studies, direct photoactivations of strychnine (glycine receptor) and muscimol (GABA receptors) have been explored further.[701,702]

As the target receptors in photoaffinity-labelling studies grow more complex and the number of photolabel peptide/protein analogues increases, the functional distinction between simple photoaffinity labelling and photocross-linking becomes less clear. However, new applications of bifunctional reagents continue to appear. For example, *p*-azido-α-diazoacetophenone has been used to crosslink pepsin internally,[703] and the subunit structure of an ATPase has been explored with a 3′-(*O*-arylazido) 8-azido ATP analogue.[704] 4-(Acetoxymercuri)-phenyl azide has been introduced as a sulphydryl-specific reagent that modified an aspartate residue in parvalbumin on irradiation.[705] A particularly interesting study of chain movements in scallop myosin during muscle relaxation/rigor utilized *p*-azidophenacyl bromide and benzophenone 4-maleimide.[706] Numerous studies have been published on the use of arylazido derivatives of peptide hormones to locate, stimulate irreversibly,[707] and study the metabolism

[697] W. Kramer, G. Burckhardt, F. A. Wilson, and G. Kurz, *J. Biol. Chem.*, 1983, **258**, 3623.
[698] P. Von Dippe, P. Drain, and D. Levy, *J. Biol. Chem.*, 1983, **258**, 8890.
[699] P. Muhn and F. Hucho, *Biochemistry*, 1983, **22**, 421.
[700] H. A. M. Ratwatte and K. T. Douglas, *Biochem. Biophys. Res. Commun.*, 1983, **112**, 273.
[701] D. Graham, F. Pfeiffer, and H. Betz, *Eur. J. Biochem.*, 1983, **131**, 519.
[702] T. Asand, J. Sakakibara, and N. Ogasawara, *FEBS Lett.*, 1983, **151**, 277.
[703] S. H. Hixson, J. L. Hurwitz, K. L. Langridge, D. C. Nichols, K. M. Provost, and A. M. Wolf, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 630.
[704] H. J. Schäfer, L. Mainka, G. Rathgeber, and G. Zimmer, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 732.
[705] K. Bose and A. Bothner-by, *Biochemistry*, 1983, **22**, 1342.
[706] P. M. D. Hardwicke, T. Wallimann, and A. G. Szent-Györgyi, *Nature (London)*, 1983, **301**, 478.
[707] E. Hazum and D. Keinan, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 1902.

of[708,709] their receptor systems. This aspect of photocrosslinking has recently been reviewed.[710,711]

Lastly, some special applications of photoaffinity techniques may be mentioned. The formation of a toxoid from *P. aeruginosa* exotoxin has been achieved by blocking the NAD^+ binding site with (3-azidopyridine)-NAD^+.[712] Photoaffinity labelling achieved inactivation without loss of antigenicity, but 4% of the toxin activity remained after repeated photolabelling. A further example of 'photosuicide' labelling has been reported.[713] A *p*-aminophenyl diazonium analogue of ouabain, which was stable in the dark, could be photoactivated to label (Na^+,K^+)-ATPase *via* a mechanism proposed to involve an aryl carbonium ion. Agarose-bound unsaturated steroid ketones have been used to photoimmobilize Δ^5-3-ketosteroid isomerase.[714]

7 Mechanism-based Inhibitors

Enzyme-activated, mechanism-based 'suicide' inhibitors have continued to attract attention both as potential therapeutic agents (for reviews see refs. 715–718, 719, and 720) and as mechanistic probes of individual enzymes.

Two important new classes of mechanism-based inactivators of serine proteases have been described. The first, haloenol derivatives of valero- and butyro-lactones,[721] were shown to inactivate chymotrypsin efficiently with turnover/inactivation ratios as low as 1.7. These compounds are essentially masked forms of α-halo ketones and presumably react with histidine in the active centre after formation of an acyl-enzyme. The second class of agent, substituted 6-chloro-2-pyrones,[722] probably function by formation of either an acyl chloride or a ketene within the active centre after ring opening by serine. The pyrone derivatives are stated to be more stable to hydrolysis under neutral or acidic conditions than are the haloenol lactones. A rearrangement from an acyl-enzyme also appears to be involved in the inactivation of a β-lactamase by

[708] A. Rashidbaigi, A. E. Ruoho, D. A. Green, and R. B. Clark, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 2849.
[709] F. M. Brennan, S. A. Grace, and C. J. Elson, *J. Immunol. Methods*, 1983, **56**, 149.
[710] T. H. Ji and I. Ji, *Biol. Reprod.*, 1983, **28**, 50.
[711] A. N. Eberle, *J. Recept. Res.*, 1983, **3**, 313.
[712] S. Marburg, R. L. Tolman, and L. T. Callahan, tert., *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 2870.
[713] M. P. Goeldner, C. G. Hirth, B. Rossi, G. Ponzio, and M. Lazdunski, *Biochemistry*, 1983, **22**, 4685.
[714] M. Hearne and W. F. Benisek, *Biochemistry*, 1983, **22**, 2537.
[715] R. H. Abeles, *Basic Life Sci.*, 1983, **25**, 286.
[716] C. T. Walsh, *Trends Biochem. Sci.*, 1983, **8**, 254.
[717] T. M. Penning, *Trends Pharmacol. Sci.*, 1983, **4**, 212.
[718] R. A. Neal, T. Sawahata, J. Halpert, and T. Kamataki, *Drug Metab. Rev.*, 1983, **14**, 49.
[719] P. R. Oritz de Montellano and M. A. Correia, *Annu. Rev. Pharmacol. Toxicol.*, 1983, **23**, 481.
[720] F. Jordan, J. F. Cohen, C. T. Wang, J. M. Wilmott, S. S. Hall, and D. L. Foxall, *Drug Metab. Rev.*, 1983, **14**, 723.
[721] S. B. Daniels, E. Cooney, M. J. Sofia, D. K. Chakravarty, and J. A. Katzenellenbogen, *J. Biol. Chem.*, 1983, **258**, 15 046.
[722] R. B. Westkaemper and R. H. Abeles, *Biochemistry*, 1983, **22**, 3256.

6-acetylmethylene penicillanic acid.[723,724] Here, it was suggested that Schiff-base formation between an enzyme amino group and the acetyl group in the acyl-enzyme could result either in enzyme regeneration or in the formation of a stable adduct of enzyme and the pyrrolothiazolidine rearrangement product.

An unusual example of the enzymatic generation of a reactive crosslinking agent has been reported in the inactivation of γ-cystathionase by trifluoromethionine.[725] It was proposed that cleavage of the thioether bond in this substrate gives rise to CSF_2, which inactivates the enzyme by formation of an internal thiourea crosslink.

The inactivation of monoamine oxidase by *trans*-2-phenyl cyclopropylamine has been reinvestigated.[726] It had been suggested previously[727] that oxidation of this compound to phenylcyclopropanone was followed by formation of a stable adduct of the cyclopropanone carbonyl with an enzyme sulphydryl. However, the only product that could be isolated from the inactivated enzyme was a derivative of cinnamaldehyde. A mechanism involving a one-electron oxidation followed by ring opening and recombination of benzylic and enzyme radicals has now been proposed. The inactivation of lactate and alcohol dehydrogenases by monocyclic and bicyclic cyclopropylmethanols such as bicyclo[4,1,0]-heptan-7-ylmethanol has also been observed.[728] In this case inactivation was thought to proceed by concomitant cyclopropane ring opening by an enzyme nucleophile and hydride transfer to NAD^+ from the zinc-co-ordinated hydroxymethyl group.

Thymidylate synthetase has often been the target of mechanism-based inactivator design because of the known clinical usefulness of one of its inhibitors, 5-fluorouracil. Several examples of inhibitors based on stabilization of the enzyme–substrate adduct at C-6 of the pyrimidine ring have been reported in recent years. A new inhibitor, 5-*p*-benzoquinone 2′-deoxyuridine 5′-phosphate, makes use of the reduction of the quinone substituent of a quinol function in order to oxidize the pyrimidine–enzyme adduct to a stable form that can no longer participate in the normal enzyme-catalysed reaction.[729]

Mechanism-based inhibitors are being used increasingly as pharmacological tools where a high degree of specificity for enzyme inhibition is required. For example, the action of α-fluoromethyl histidine on histidine decarboxylase has been shown to prolong the antisecretory action of the H_2-receptor antagonist cimetidine[730] and also reduces the uptake of histidine by rat mast cells.[731] Expectations of inhibitor specificity *in vivo* are not always met, however, and

[723] M. Arisawa and S. Adam, *Biochem. J.*, 1983, **211**, 447.
[724] M. Arisawa and R. Then, *Biochem. J.*, 1983, **209**, 609.
[725] R. W. Fuller, S. K. Hemrick-Luecke, and B. B. Molloy, *Biochem. Pharmacol.*, 1983, **32**, 1243.
[726] R. B. Silverman, *J. Biol. Chem.*, 1983, **258**, 14 766.
[727] C. Paech, J. I. Salach, and T. P. Singer, *J. Biol. Chem.*, 1983, **255**, 2700.
[728] I. MacInnes, D. C. Nonhebel, S. T. Orszucik, C. J. Suckling, and R. Wrigglesworth, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2771.
[729] L. Maggiora, C. T. C. Chang, M. E. Hasson, C. F. Bigge, and M. P. Mertes, *J. Med. Chem.*, 1983, **26**, 1028.
[730] M. Bouclier, M. J. Jung, and F. Gerhart, *Biochem. Pharmacol.*, 1983, **32**, 1553.
[731] M. T. Bauza and D. Lagunoff, *Biochem. Pharmacol.*, 1983, **32**, 59.

β-methylene aspartate, a potent inhibitor of glutamate oxaloacetate decarboxylase, has been shown to produce major alterations in cysteine sulphinate metabolism because the inhibitor also inactivates cysteine sulphinate decarboxylase.[732]

Suicide or paracatalytic inactivation of the pyruvate dehydrogenase complex has been the subject of several reports, but the mechanisms involved are diverse. Branched-chain keto acids inactivate the bovine-heart enzyme, and in the case of 2-keto isovalerate this is associated with slow formation of an isobutyryl lipoate derivative of the enzyme.[733] However, modification of the cofactor thiamine pyrophosphate is implicated in paracatalytic inactivation by pyruvate of the pig-heart enzyme.[734] Formation of a stable adduct with the cofactor also examples the activity of (*E*)-4-(4-chlorophenyl)-2-oxo-3-butenoic acid against the yeast enzyme.[735] The inactivation of asparagine *N*-glycosyltransferases by epoxide-containing peptides[736] appeared to be an example of affinity labelling, but it was found to be enzyme-activated with irreversible inhibition occurring only when glycosyl transfer occurred.

PART II: *X*-Ray Studies

By W. D. Mercer

1 Introduction

The literature published in 1983 has again shown the increasing range of systems being examined by *X*-ray or neutron-diffraction methods. A large number of preliminary crystallization reports have appeared, and these are presented in Table 1.

The increasing use of synchrotron radiation sources is reflected not only in the number of structures where the data were collected using such sources but also in an increasing number of reports of equipment or theoretical considerations related to synchrotron radiation. Again this year the use of computer graphics has increased and a new journal devoted to molecular graphics has appeared.

A wide and interesting range of new high-resolution structures has appeared in the literature. Of particular interest are the structures of Bence-Jones protein Rhe, human oxyhaemoglobin, azurin, kallikrein, glutathione reductase, and a redetermination of phospholipase A2. Several virus structures have been described together with the structures of neuraminidase and haemagglutinin derived from the influenza virus. Interesting structural comparisons of the small, spherical plant virus group have also been presented.

[732] O. W. Griffith, *J. Biol. Chem.*, 1983, **258**, 1591.
[733] R. H. Jackson and T. P. Singer, *J. Biol. Chem.*, 1983, **258**, 1857.
[734] B. Sümegi and I. Alkonyi, *Arch. Biochem. Biophys.*, 1983, **223**, 417.
[735] D. J. Kuo and F. Jordan, *Biochemistry*, 1983, **22**, 3735.
[736] E. Bause, *Biochem. J.*, 1983, **209**, 323.

Table *Preliminary crystallization reports*

Protein	*Source*	*Space group*	*Cell dimensions*				*Mol. wt. and no. of subunits*	*Mol. wt. of asymmetric unit*	V_M/nm^3 dalton^{-1} × 10^3	*Precipitant*	pH	*Ref.*
			a/nm	*b*/nm	*c*/nm	*β*/°						
Retinol-binding protein	Human plasma	*R*3	10.42	10.42	7.45	–	21 000(1)	–	–	Sodium chloride	6.8	*a*
Matrix porin	*E. coli*	$P4_2$	15.5	15.5	17.2	–	110 000(3)	220 000	4.69	PEG4000[c]	7	*b*
		$P6_322$	9.3	9.3	22.0	–	110 000(3)	37 000	3.76	PEG4000	–	*b*
GTP:AMP phosphotransferase	Beef-heart mitochondria	*I*4	8.82	8.82	14.78	–	26 000(1)	52 000	2.76	PEG6000	8.6	*d*
Plastocyanin	*Enteromorpha prolifera*	*I*4	5.39	5.39	5.94	–	10 500(1)	10 500	2.05	Ammonium sulphate	7.0	*e*
Plastocyanin	*Cucumis sativa*	$P4_1$ or $P4_3$	6.67	6.67	4.60	–	10 500(1)	21 000	2.44	Ammonium sulphate	7–8.5	*e*
Fab fragment monoclonal anti-digoxin antibody[f]	–	$P2_1$	5.91	8.91	4.38	96.3	50 000(1)	50 000	2.29	MPD[g]	5.7	*h*
Fab fragment monoclonal anti-phenyl arsonate antibody	–	$P2_1$	8.62	8.04	7.58	90.3	50 000(1)	100 000	2.63	Ammonium sulphate	6–7	*i*
Protein Riv[j]	Human	$P2_12_12_1$	8.01	14.55	5.01	–	54 000(2)	54 000	2.70	Sodium chloride	7.0	*k*
Lipoamide dehydrogenase	*Azotobacter vinelandii*	$P2_12_12_1$	6.42	8.38	19.3	–	112 000(2)	112 000	2.3	PEG4000	7.5	*l*
Mitogenic lentil lectin	*Lens culinaris*	$P2_12_12_1$	5.01	6.78	13.02	–	47 000 ($\alpha_2\beta_2$)	47 000	2.35	PEG4000	5.5	*m*
Ribonuclease[n]	Bovine pancreas	$P3_221$	6.77	6.77	6.51	$\gamma = 120°$	14 000(1)	14 000	3.00	Ammonium sulphate	5.2	*p*
Ribonuclease[o]	Bovine pancreas	$P3_221$	6.72	6.72	6.47	$\gamma = 120°$	14 000(1)	14 000	3.00	Ammonium sulphate	5.2	*p*
Haemocyanin 94K subunit	*Panulirus interruptus*	$P6_322$	19.56	19.56	15.80	–	500 000(6)	500 000	4.9	Ammonium sulphate	5.5	*q*
Serine proteinase inhibitor CI-2	Barley seeds	*P*622	6.91	6.91	5.29	–	9100 (1)	9100	2.0	Ammonium sulphate	8.0	*r*
Elongation factor G	*Thermus thermophilus*	$P2_12_12_1$	7.62	10.76	11.71	–	76 000	76 000	3.08	MPD	7.8	*s*, *t*
cro repressor–DNA complexes	Phage λ	$C222_1$	8.11	8.92	8.00	–	45 000(2)	45 000	3.2	PEG6000	4.0	*u*
F_c fragment monoclonal anti-*p*-azophenyl arsonate antibody (R19.9)	Mouse	$P4_1$ or $P4_3$	13.43	13.43	14.40	–	50 000(2)	50 000	3.2	Sodium citrate	8–8.5	*v*

Table (*cont.*)

Protein	*Source*	*Space group*	*Cell dimensions*				*Mol. wt. and no. of subunits*	*Mol. wt. of asymmetric unit*	V_M/nm^3 dalton^{-1} × 10^3	*Precipitant*	pH	*Ref.*
			a/nm	*b*/nm	*c*/nm	*β*/°						
Ascorbate oxidase	*Cucurbita pepo medullosa*	$P2_12_12$	10.67	10.51	11.35	–	140 000(2)	140 000	2.27	MPD	5.6	*w*
Fab fragment monoclonal anti-*p*-phenyl arsonate antibody (R9.3)	–	$P2_1$	9.83	6.75	6.69	108.4	50 000(1)	100 000	2.11	PEG6000	6.0	*x*
	–	$P4_1$ or $P4_3$	4.59	4.59	23.29	–	50 000(1)	50 000	2.45	PEG6000 + sodium chloride	6.0	*x*
Creatine kinase	Bovine heart	$P2_12_12$	13.3	12.8	6.5	–	80 000(2)	80 000	3.45	MPD	7.2	*y*
		$P4_22_12$	13.2	13.2	7.5	–	80 000(2)	40 000	3.8	MPD	7.2	*y*
Trichorzianine A1^z	*Trichoderma harzianum*	$C222_1$	6.48	0.933	3.99	–	2100(1)	2100	–	Evapoation	–	*aa*
Single-strand DNA-binding protein	*E. coli*	$C2$	10.55	6.27	9.81	112.6	75 500(4)	*bb*	2.2	Sodium chloride	9	*cc*
		$P6_222$ or $P6_422$	6.14	6.14	26.43	–	75 500(4)	–	2.4	Sodium chloride	9	*cc*
Fab fragment monoclonal antilysozyme antibody + lysozyme	–	$P2_1$	5.55	14.35	4.91	120.33	–	–	–	PEG6000	6–7	*dd*
Col E1 Rop protein	–	$C2$	5.73	4.02	2.72	103	17 000(2)	8500	2.14	MPD	7.4	*ee*
Rubredoxin	*Desulfovibrio desulfuricans*	$P1$	2.492	1.779	1.972	$\alpha = 101.0°$ $\beta = 83.4°$ $\gamma = 104.5°$	5500(1)	5500	1.55	Ammonium sulphate	4.0	*ff*
Ribosomal protein L25 + ribonuclease-resistant fragment of 5S RNA[gg]	*E. coli*	$P6_122$ or $P6_522$	11.9	11.9	25.0	–	–	–	2.1	MPD	7.0	*hh*
D-Glyceraldehyde 3-phosphate dehydrogenase	*Palinurus versicolor*	$C2$	12.77	9.96	8.05	114.1	144 000(4)	72 000	3.27	Ammonium sulphate	6.1	*ii*
Glucose 6-phosphate dehydrogenase	Rat liver	$I222$ or $I2_12_12_1$	6.60	14.08	17.78	–	116 000(2)	116 000	1.78	Ammonium sulphate	7	*jj*
β-Lactamase	*Enterobacter cloacae* P99	$P2_12_12$	7.74	6.94	6.36	–	39 000(1)	39 000	2.19	PEG	7	*kk*
Citrate synthase	*E. coli* B	$P23$	22.0	22.0	22.0	–	170 000(4)	340 000	2.57	Ammonium sulphate	7.0	*ll*

Phaseolin	*Phaseolus vulgaris*	*P*432	6.7	6.7	6.7	–	150 000(3)	–	–	Ammonium sulphate	6.7	*mm*
		$P2_12_12$	12.8	13.6	16.2	–	150 000(3)	–	–	Ethanol	6.7	*mm*
		$P2_12_12_1$	11.3	13.6	8.9	–	150 000(3)	–	–	Ethanol	6.7	*mm*
Ovalbumin	Hen	*C*2	11.65	4.18	7.05	101.5	52 000(1)	52 000	2.0	Ammonium sulphate	5.4	*nn*
Ovalbumin	Hen	*P*1	6.20	8.43	7.22	$\alpha = 87.4°$ $\beta = 103.9°$ $\gamma = 108.7°$	52 000(1)	–	–	Ammonium sulphate	5.8	*nn*
Plakalbumin[oo]	Hen	*C*2	9.99	10.20	7.93	112.1	52 000(1)	103 500	1.81	Ammonium sulphate	5.4	*nn*
Glucose 6-phosphate dehydrogenase	*Leuconostoc mesenteroides*	$P3_121$ or $P3_221$	10.58	10.58	22.51	–	106 000(2)	106 000	3.4	Phosphate	5.8	*pp*
Cadmium, zinc metallothionein	Rat liver	$P4_12_12$ or $P4_32_12$	3.10	3.10	12.00	–	6500(1)	6500	2.2	Sodium formate	7.5	*qq*
S-Adenosylmethionine synthetase	*E. coli*	$P6_222$ or $P6_422$	12.88	12.88	14.00	–	180 000(4)	45 000	3.73	–	–	*rr*
		$P4_12_12$ or $P4_32_12$	12.18	12.18	17.26	–	180 000(4)	90 000	3.55	–	–	*rr*
Aspartyl tRNA synthetase–tRNA complex	Yeast	*I*432	35.4	35.4	35.4	–	125 000(2)	175 000	5.3	Ammonium sulphate	7.8	*ss*
Trypsin inhibitor E-I	Soybean	*F*432	12.8	12.8	12.8	–	8000(1)	8000	2.85	PEG4000	4.6	*tt*
		$P4_132$ or $P4_332$	8.71	8.71	8.71	–	8000(1)	16 000	1.80	PEG4000	4.6	*tt*
P_{II} regulatory protein	*E. coli*	*I*23	8.88	8.88	8.88	–	44 000(4)	11 000	2.65	Ammonium sulphate	7.6	*uu*
Riboflavin synthase	*Bacillus subtilis*	$P6_322$	15.64	15.64	29.85	–	1 000 000 $(3\alpha + 6\beta)$	1 000 000	3.17	Phosphate	8.7	*vv*
Trp-repressor: L-tryptophan	*E. coli*	$P3_121$ or $P3_221$	5.03	5.03	7.36	–	12 500	12 500	2.48	Phosphate	5.35	*ww*
		$P4_122$ or $P4_322$	8.17	8.17	7.52	–	12 500	25 000	2.51	Ammonium sulphate	6.2	*ww*
Trp-repressor: indole 3-propionate	*E. coli*	$P4_122$ or $P4_322$	8.17	8.17	7.52	–	12 500	25 000	2.51	Ammonium sulphate	6.2	*ww*
Nitrogenase iron protein	*Azotobacter vinelandii*	$P2_12_12$	9.46	17.99	7.41	–	63 200(2)	–	–	MPD	7–8	*xx*

Table (*cont.*)

Protein	*Source*	*Space group*	*Cell dimensions*				*Mol. wt. and no. of subunits*	*Mol. wt. of asymmetric unit*	V_M/nm^3 dalton^{-1} × 10^3	*Precipitant*	pH	*Ref.*
			a/nm	*b*/nm	*c*/nm	*β*/°						
Ribosomal protein L30	*Bacillus stearothermophilus*	$P4_12_12$ or $P4_32_12$	4.63	4.63	6.14	–	7053(1)	7053	2.33	–	–	*yy*
Ribosomal protein S5	*Bacillus stearothermophilus*	$P3_121$ or $P3_221$	5.93	5.93	10.98	–	17600(1)	17600	3.2	–	–	*yy*
Ribulose 1,5-bisphosphate carboxylase/oxygenase[zz]	Spinach	$P2_12_12$	18.4	21.8	11.9	–	560000 (8 + 8)	280000	2.1	PEG6000	5–6	*aaa*
		*I*422	27.5	27.5	17.8	–	560000 (8 + 8)	280000	3.1	PEG6000	5–6	*aaa*
50S ribosomal particles	*Bacillus stearothermophilus*	–	–	–	–	–	–	–	–	–	–	*bbb*
Proteinase II	*Crotalus adamantus*	$P3_112$ or $P3_212$	7.33	7.33	9.58	–	24000(1)	–	–	Ammonium sulphate	5	*ccc*
Anti-T lectin	Peanut	$P2_1$	7.03	10.11	8.45	114.0	110000(4)	110000	2.49	PEG6000	4.6	*ddd*
		$P2_1$	7.05	10.73	8.50	114.0	110000(4)	110000	2.67	PEG6000	4.6	*ddd*
		*P*1	5.20	7.11	8.36	$\alpha = 65.4°$ $\beta = 77.1°$ $\gamma = 71.5°$	110000(4)	110000	2.7	PEG6000	4.6	*ddd*
Ribosomal protein L6	*Bacillus stearothermophilus*	$P6_122$ or $P6_522$	7.27	7.27	12.49	–	19000(1)	19000	2.4	Phosphate	7.6–8.2	*eee*
50S ribosomal subunits	*Bacillus stearothermophilus*	–	–	–	–	–	–	–	–	–	–	*fff*
Cytochrome bc_1 complex	Beef-heart mitochondria	–	11.3	11.3	13.2	–	–	–	–	–	–	*ggg*
Rhinovirus[hhh]	–	$P2_12_12$	32.3	35.8	38.0	–	8.4×10^6	–	2.5	Ammonium sulphate	7.2	*iii*

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Synchrotron sources have also been used in the study of the time course of muscle contraction, and the use of neutron scattering to determine the shapes of ribosomal proteins *in situ* has yielded results.

Once again space considerations have precluded detailed presentation of amino acid structures, but a section on peptide and amino acid structures is included. These include [Leu^5]enkephalin together with constituent fragments of enkephalin, gramicidin A, neurotensin, substance P, and adrenocorticotropic hormone.

2 Crystallographic Theory and Methods

Theory and Methods. – Schenk[1] has reviewed some of the recent developments in direct methods for solving crystal structures, and Piro[2] has discussed the crystallographic-phase problem in terms of information theory. Techniques for random phase refinement have been described,[3] and a procedure for the automatic determination of crystal structures having pseudo-translational symmetry has been reported.[4]

The application of direct methods to difference structures, in particular non-centrosymmetric structures containing heavy atoms, has been published,[5] and an alternative to direct methods, a real-space Patterson search method, has been described and tested.[6]

The new sorts of magnets that will lead to enhanced synchrotron radiation beams have been reported,[7] and the early problems with two of the new synchrotron sources have been described.[8] Glatter and Laggner[9] have analysed the possibility of using polychromatic synchrotron radiation for *X*-ray small-angle experiments, and Russell[10] has described the methodology of determining the incident-beam intensity for small-angle *X*-ray scattering measured with position-sensitive detectors.

The use of very low-angle glancing *X*-ray experiments to study the surface structure of two-dimensional systems has been described.[11] Uzgiris and Kornberg[12] have shown how two-dimensional crystals can be produced.

Shmueli and Wilson[13] have considered the generalized intensity statistics for structures with partial centrosymmetry, and Post[14] has described the experimental

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determination of the phases of *X*-ray reflections. Jauch[15] has considered the effect of random errors on partially phased Fourier maps. Two sources of error, inaccuracy in the atomic co-ordinates used in the phasing model and errors in the observed structure-factor amplitudes, are analysed.

Duisenberg[16] has described both anisotropic mosaicity and splitting of a crystal in terms of a rotational cluster model and also the application to data collection on a diffractometer system. Clegg[17] has described a reliable and automatic method for the refinement of cell and orientation parameters in four-circle diffractometry, and Axe and Hastings[18] have reported expressions for the Lorentz factors for crystals with a large mosaic spread. A simple method for the determination of Bravais lattices has been described,[19] and three corrections to 'International Tables for *X*-Ray Crystallography' have been reported.[20–22]

Baur and co-workers[23] have described the topological analysis of crystal structures to study the topological symmetry of complex ionic compounds.

Equipment. — Kulda and Mikula[24] have described a medium-resolution double-crystal diffractometer for the study of small-angle neutron scattering. The equipment causes a gain both in intensity and in signal-to-noise ratio.

Milch has reported[25] the design of a focusing *X*-ray camera used to record low-angle diffraction from small specimens. A fast *X*-ray shutter and simple ion chambers useful for alignment and operation are also described.

Sakabe has described[26] a modification of a conventional Weissenberg camera for use in macromolecular crystallography. The system includes a lithium fluoride monochromator, a newly designed Weissenberg goniometer with multi-layer line screens, and a controller. Compared to an oscillation camera a larger proportion of the reflections are fully recorded.

A remote-control direct-beam exposure device for the Arndt–Wonacott rotation camera has been reported.[27] The use of this equipment is particularly useful with synchrotron *X*-ray sources.

An automated control system for the independent operation of two radiation shutters on a rotating-anode *X*-ray source has been described,[28] and a simple way of cutting a crystal in any desired direction has been reported.[29]

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[27] J. R. Helliwell and A. W. Thompson, *J. Appl. Crystallogr.*, 1983, **16**, 579.
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Data Processing and Collection. – Rossmann and Erickson[30] have described a method for determining the accurate setting of a crystal placed in an unknown orientation on an oscillation camera. The method avoids the usual setting operations and so avoids radiation damage, whilst also maximizing the use of synchrotron radiation time.

Brown has described[31] the crystallographic file structure that has been developed by the International Union of Crystallography. It is intended that this standardized file will allow portability of data between program systems or laboratories.

Trueblood and Dunitz have examined[32] the possibility of determining information about the internal motion in crystals from *X*-ray or neutron studies and have shown that it can unambiguously resolve different modes of internal motions in a given molecule.

A method that allows the collection of 100–150 reflections per hour on a diffractometer has been described.[33] The method gives no significant loss in accuracy, and the authors have also determined the homogeneity of the monochromated primary beam.

The processing of oscillation camera data to consider the size and shape of diffraction spots produced by a monochromatized synchrotron radiation source has been described.[34] The authors also establish a theoretical basis for the procedures to be used in a polychromatic diffraction experiment.

Walker and Stuart have described[35] an empirical method for correcting diffractometer data. The method requires no knowledge of the crystal dimensions or the linear absorption coefficient and needs no extra data beyond the unique set. The authors compare the method with other commonly used corrections.

Shakked has described[36] an anisotropic scaling method for three-dimensional data, and Lewis and Rees[37] have shown that the statistical methodology introduced for the treatment of negative-intensity observations can be used to estimate anomalous-scattering differences.

A method for determining the positions, occupancies, and thermal parameters of heavy-atom sites has been presented.[38] The technique uses an origin-removed Patterson function, and theoretical and practical aspects of the method are considered.

Taylor and Kennard[39] have examined the estimation of average molecular dimensions from crystallographic data. They consider in detail the statistical rationale for determining such mean dimensions and have applied the method to the structures in the Cambridge Structural Database.

[30] M. G. Rossmann and J. W. Erickson, *J. Appl. Crystallogr.*, 1983, **16**, 629.
[31] I. D. Brown, *Acta Crystallogr., Sect. A*, 1983, **39**, 216.
[32] K. N. Trueblood and J. D. Dunitz, *Acta Crystallogr., Sect. B*, 1983, **39**, 120.
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[34] T. J. Greenhough, J. R. Helliwell, and S. A. Rule, *J. Appl. Crystallogr.*, 1983, **16**, 242.
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[38] T. C. Terwilliger and D. Eisenberg, *Acta Crystallogr., Sect. A*, 1983, **39**, 813.
[39] R. Taylor and O. Kennard, *Acta Crystallogr., Sect. B*, 1983, **39**, 517.

Protein Crystallographic Techniques. – Mowbray and Petsko[40] have described how heavy-atom binding sites can be introduced into crystalline proteins. Treatment with carbon disulphide, under anaerobic conditions, followed by exposure to mercurials will derivatize amino groups on the protein, and by carrying out the reaction on crystals the number of sites of reaction is limited.

A novel mercurial agent, methylphenylmercury, has been reported.[41] The compound reacts with two usually inaccessible cysteines in crystals of carbonmonoxyhaemoglobin but does not react with a third, usually reactive, cysteine and may therefore be useful in preparing new heavy-atom derivatives.

Wilson and co-workers[42] have described the collection of three-dimensional data from protein crystals using synchrotron radiation. Exposure times are reduced fifty-fold and crystal life is extended at least five-fold when compared with a conventional rotating-anode source.

Nikonov[43] has reported a correct calculation of the scale factor between isomorphous *X*-ray data based on a statistical criterion. This scale factor requires no additional refinement, improves the signal-to-noise ratio of difference Patterson maps, and allows an estimation of the degree of non-isomorphism.

Cannillo and co-workers[44] have described a phase-extension method that uses density modification, and Schoenborn[45] has analysed peak-shapes with position-sensitive detectors in protein neutron crystallography.

Structure Refinements. – Rees and Lewis[46] have shown how the experimental phase information can be incorporated into a restrained least-squares refinement, thereby increasing the ratio of observed data to refined parameters.

Rayment[47] has considered the optimum strategy and inherent limitations in the application of the molecular replacement method at low resolution, using calculations on icosahedral viruses as a test system.

Bhat and Blow[48] have described a method for refining partially interpreted protein structures and include a procedure for scaling between a model and an electron-density map.

Morffew and Moss[49] have described a strategy for combining restrained least-squares with the use of computer graphics in the refinement of protein structures. A weight can be applied to the shifts based on the confidence in the atomic positions derived from the graphics system.

Herzberg and Sussman[50] have reported the use of a macromolecular model-building program that fits a set of constrained groups to a set of approximate

[40] S. L. Mowbray and G. A. Petsko, *J. Biol. Chem.*, 1983, **258**, 5634.
[41] D. J. Abraham, S. E. V. Phillips, and P. E. Kennedy, *J. Mol. Biol.*, 1983, **170**, 249.
[42] K. S. Wilson, E. A. Stura, D. L. Wild, R. J. Todd, D. I. Stuart, Y. S. Babu, J. A. Jenkins, T. S. Standing, L. N. Johnson, R. Fourme, R. Kahn, A. Gadet, K. S. Bartels, and H. D. Bartunik, *J. Appl. Crystallogr.*, 1983, **16**, 28.
[43] S. V. Nikonov, *Acta Crystallogr., Sect. A*, 1983, **39**, 693.
[44] E. Cannillo, R. Oberti, and L. Ungaretti, *Acta Crystallogr., Sect. A*, 1983, **39**, 68.
[45] B. P. Schoenborn, *Acta Crystallogr., Sect. A*, 1983, **39**, 315.
[46] D. C. Rees and M. Lewis, *Acta Crystallogr., Sect. A*, 1983, **39**, 94.
[47] I. Rayment, *Acta Crystallogr., Sect. A*, 1983, **39**, 102.
[48] T. N. Bhat and D. M. Blow, *Acta Crystallogr., Sect. A*, 1983, **39**, 166.
[49] A. J. Morffew and D. S. Moss, *Acta Crystallogr., Sect. A*, 1983, **39**, 196.
[50] O. Herzberg and J. L. Sussman, *J. Appl. Crystallogr.*, 1983, **16**, 144.

guide co-ordinates. Simultaneously the program constrains the stereochemistry between groups.

Rees[51] has calculated the values of the *R*-factor for structures calculated by non-crystallographic symmetry averaging and phase requirement. It is shown that in all cases, even incorrectly positioned non-crystallographic symmetry elements, the *R*-factor will be less than the value of 0.586 characteristic of wrong structures lacking non-crystallographic symmetry.

Computer Graphics. – A quarterly journal dedicated to graphics, *The Journal of Molecular Graphics*, has appeared in 1983 and presents papers on both hardware and software of relevance to the study of proteins and peptides.

An interactive program for simulating protein receptor and substrate interactions has been described.[52] The system uses an Evans and Sutherland Picture System 2 and in additon to displaying structures can perform energy refinement in a non-interactive mode.

A computer program to translate PLUTO plotting instructions into those usable by ORTEP has been reported.[53] The advantages are the more understandable PLUTO instructions with the higher-quality pictures produced by ORTEP.

The measurement of co-ordinates from a Kendrew model using two theodolites has been described,[54] and a way of determining the co-ordinates from two projections of a Kendrew model has been reported.[55]

Connolly has described[56] a computer algorithm for calculating the part of the van der Waals surface of a molecule that is accessible to solvent, and he has shown how the results can be displayed on either vector or raster computer-graphics systems.

Bash and co-workers[57] have described a similar algorithm that generates the molecular surface on a real-time graphics system. Bond rotations and global transformations can be accommodated, and the system will provide a powerful way of performing real-time molecular modelling.

3 Immunoglobulins

Bence-Jones Protein, Rhe. – The structure of a novel Bence-Jones fragment has been reported at 0.16 nm resolution.[58] The protein, Rhe, is a λ-type Bence-Jones protein fragment and exists in the crystal, and in solution, as a dimer. The 'immunoglobulin fold' is generally preserved but shows significant differences from other Bence-Jones proteins or Fab fragments in terms of both the monomer conformation and the association of monomers into dimers. Some of the changes in the monomer include non-hypervariable residues that had been thought

[51] D. C. Rees, *Acta Crystallogr., Sect. A*, 1983, **39**, 916.
[52] B. Busetta, I. J. Tickle, and T. L. Blundell, *J. Appl. Crystallogr.*, 1983, **16**, 432.
[53] G. Bandel and J. L. Sussman, *J. Appl. Crystallogr.*, 1983, **16**, 650.
[54] L. Lebioda, M. H. Hatada, and A. Tulinsky, *J. Appl. Crystallogr.*, 1983, **16**, 270.
[55] P. Dumas and B. Rees, *J. Appl. Crystallogr.*, 1983, **16**, 658.
[56] M. I. Connolly, *J. Appl. Crystallogr.*, 1983, **16**, 548.
[57] P. A Bash, N. Pattabiraman, C. Huang, T. E. Ferrin, and R. Langridge, *Science*, 1983, **222**, 1325.
[58] W. Furey, jun., B. C. Wang, C. S. Yoo, and M. Sax, *J. Mol. Biol.*, 1983, **167**, 661.

to be part of a structurally invariant domain. The previously unseen mode of dimerization also gives rise to combining site geometries unobtainable with the conventional mode of interaction. The structure has been determined very accurately, giving a crystallographic residual (R_f) value of 0.149, and refinement has allowed the positioning of several structurally important water molecules, some of which appear essential to stabilize the novel conformation.

Immunoglobulin G. – Sutton and Phillips[59] have described the structure of the carbohydrate within the Fc fragment of immunoglobulin G. The two heavy chains in the Fc molecule fold into two domains, each consisting of a 'sandwich' of two antiparallel β-sheets. The carbohydrate can be seen clearly attached to an asparagine residue (Asn-297) and extends into the space between the two C_H2 domains. A ten-residue branched carbohydrate chain has been fitted to the electron density and is seen to adopt a different conformation on the two chains of the Fc molecule.

4 Oxygen- and Electron-carrying Proteins

Haemoglobin. – Shaanan has reported[60] the structure of human oxyhaemoglobin at 0.21 nm resolution, which has been refined to a final *R*-factor of 0.223. The active sites are seen to be significantly different in the α- and β-subunits. The iron atoms are 0.016 nm and 0.000 nm from the mean plane of the porphyrin carbon and nitrogens in the α- and β-subunits, respectively, the porphyrin ring being planar in the α-subunit but ruffled in the β-subunit. The Fe–O1–O2 angles also vary, 153° in the α-subunit and 159° in the β-subunit, and the oxygen molecule forms a hydrogen bond to N^{ϵ} of HisE7 in the α-subunit but does not make this bond in the β-subunit. Bond lengths and angles agree well with those seen in oxymyoglobin and model compounds.

In other areas of the structure the two C-terminal residues are relatively delocalized and are positioned in such a way that they cannot form the inter-subunit salt bridge that is formed in deoxyhaemoglobin. The penultimate tyrosine in the β-chain shows a major and a minor location, and it appears that the previously suggested role for this residue in allosterism may have to be amended. The quaternary structure of oxyhaemoglobin is identical to that of the previously determined carbon monoxide–haemoglobin complex.

The tertiary structural changes on ligand binding to haemoglobin and their role in the allosteric function of the molecule have been described.[61] An allosteric core consisting of the haem, histidine F8, the FG corner, and part of the F helix plays a central role in co-operativity.

The structure of haemoglobin Barcelona has been determined[62] by a difference electron-density map at 0.32 nm resolution. The modification, Asp FG1 β changed to a histidine, can be clearly seen in the map, and several other discrete

[59] B. J. Sutton and D. C. Phillips, *Biochem. Soc. Trans.*, 1983, **11**, 130.
[60] B. Shaanan, *J. Mol. Biol.*, 1983, **171**, 31.
[61] B. R. Gelin, A. W.-M. Lee, and M. Karplus, *J. Mol. Biol.*, 1983, **171**, 489.
[62] S. E. V. Phillips, M. F. Perutz, C. Poyart, and J. Wajeman, *J. Mol. Biol.*, 1983, **164**, 477.

changes are seen in the molecule. An additional fixed water molecule has also been identified.

The binding of two known antilipoprotinaemia drugs, clofibrate and gemfibrozil, that stop the gelling of sickle haemoglobin has been examined.[63] Three pairs of molecules of clofibrate are bound to the walls of the internal cavity of deoxyhaemoglobin A. One pair is bound to a totally different site, between helices A, E, and H of the α-chains of carbon monoxide haemoglobin A. Unlike other antisickling agents, clofibric acid and its related compounds decrease rather than increase the oxygen affinity of haemoglobin.

Alter[64] has compared the solution and crystal structures of horse and human haemoglobins by photoacoustic spectroscopy. The majority of states of haemoglobin show no differences in the two states, but slight, and possibly functionally significant, differences are seen for both fluoride and aquomethaemoglobin.

Haemerythrin. – The structure determination of trimeric haemerythrin has been reported,[65] the structure having been solved using anomalous-scattering information from the iron atoms native to the protein. The protein monomer adopts the usual haemerythrin conformation of a four-helix bundle surrounding the di-iron centre. The association of the three monomers shows a topology different from that seen in the dimeric packing of helices and consists of a parallel mode of association.

Stenkamp and co-workers have described[66] the refinement of met- and azidomet-haemerythrin at 0.2 nm resolution. The refined structure shows variation in Fe—X bonds: Fe—N and Fe—$O_{carboxy}$ bonds are longer than 0.2 nm whereas Fe—$O_{\mu\text{-}oxo}$ bonds are shorter than 0.2 nm.

Flavodoxin. – The structure of flavodoxin from *Anacystis nidulans* has been determined at 0.25 nm resolution.[67] The polypeptide backbone of 167 residues folds into the familiar β-α-β conformation with five parallel strands in the central β-sheet. Comparison with flavodoxin from *Clostridium* MP shows that the extra 29 residues of the *Anacystis* enzyme are accommodated in a major insertion of 20 residues that form an extra lobe, the other nine being additions to external segments. Those residues added between the fourth sheet strand and the beginning of the third helix are seen to change the environment of the pyrimidine end of the flavin mononucleotide ring. Two residues, possibly tryptophans, enclose the isoalloxazine ring, which is tilted relative to its orientation in *Clostridium* MP flavodoxin. Some of the interactions show structural analogy with those seen in flavodoxin from *Desulfovibrio vulgaris*. In an appendix[68] Tarr reports the determination of the amino acid sequence of the insertion region.

[63] D. J. Abraham, M. F. Perutz, and S. E. V. Phillips, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 324.
[64] G. M. Alter, *J. Biol. Chem.*, 1983, **258**, 14966.
[65] J. L. Smith, W. A. Hendrickson, and A. W. Addison, *Nature (London)*, 1983, **303**, 86.
[66] R. E. Stenkamp, L. C. Sieker, and L. H. Jensen, *Acta Crystallogr., Sect. B*, 1983, **39**, 697.
[67] W. W. Smith, K. A. Pattridge, M. L. Ludwig, G. A. Petsko, D. Tsernoglou, M. Tanaka, and K. T. Yasunobu, *J. Mol. Biol.*, 1983, **165**, 737.
[68] G. E. Tarr, *J. Mol. Biol.*, 1983, **165**, 754.

Ferredoxin. – The sequence of 7 Fe ferredoxin from *Azotobacter vinelandii* has been determined and confirmed by an *X*-ray-diffraction study.[69] The molecule shows several differences from the clostridial-type ferredoxins, being 106 residues long against 55–60 for the other bacterial ferredoxins. The *Azotobacter* protein has nine cysteines, one of which is not homologous with other ferredoxins, and other sequence features are compared for the various ferredoxin groups.

Plastocyanin. – Poplar plastocyanin in the oxidized state has had its structure refined at 0.16 nm resolution.[70] The final *R*-factor is 0.17 with an estimated root-mean-square deviation in atom positions of 0.01 nm. The copper-atom geometry is a distorted tetrahedron with the Cu–N(His) bond lengths being 0.210 and 0.204 nm and the Cu–S(Cys) bond length being 0.213 nm. However, the Cu–S(Met) bond length of 0.290 nm is longer than expected and raises questions about its significance. Forty-four water molecules have been located.

The pH 4.2 structure has also been refined and compared with the pH 6.0 structure. There are few significant changes, and in particular the geometry of the copper site is unaffected. The paper also considers the distribution and function of the conserved residues in the large number of plastocyanins that have been sequenced.

Azurin. – The structure of the blue copper protein azurin isolated from *Alcaligenes denitrificans* has been reported at 0.3 nm resolution.[71] The two independent molecules in the asymmetric unit were interpreted independently and are related by an approximate diad axis. After partial refinement, the two molecules show no major conformational differences. The structure contains an eight-stranded β-barrel, whose topology is the same as that of plastocyanin, and a previously reported alternative folding for *Pseudomonas aeruginosa* azurin. The copper atom forms three short bonds, two to histidine nitrogen atoms and one to a cysteine sulphur atom, and one long bond, to a methionine sulphur atom, the four ligands forming a distorted tetrahedron. There is a possible fifth interaction with the carbonyl oxygen of a glycine residue. A surface hydrophobic patch has been located, which surrounds an imidazole ring, and it seems to be the most likely electron-transfer locus. The authors have also aligned the sequences of azurin and plastocyanin and discuss the observed homology.

Cytochrome *c*. – The 0.2 nm resolution structure of rice-embryo ferricytochrome *c* has been described.[72] The molecule consists of 111 residues and, compared to animal cytochromes *c*, shows the typical plant cytochrome *c* extension of eight residues at the N-terminus. These residues display a collagen-like conformation and an irregular reverse turn and are located on the surface of the molecule, near the C-terminal α-helix, by hydrogen bonds. Except for these eight residues,

[69] J. B. Howard, T. W. Lorsbach, D. Ghosh, K. Melis, and C. D. Stout, *J. Biol. Chem.*, 1983, **258**, 508.
[70] J. M. Guss and H. C. Freeman, *J. Mol. Biol.*, 1983, **169**, 521.
[71] G. E. Norris, B. F. Anderson, and E. N. Baker, *J. Mol. Biol.*, 1983, **165**, 501.
[72] H. Ochi, Y. Hata, N. Tanaka, M. Kakudo, T. Sakurai, S. Aihara, and Y. Morita, *J. Mol. Biol.*, 1983, **166**, 407.

the conformation is essentially identical to that of tuna cytochrome *c* apart from minor alterations at two positions.

Flavocytochrome b_2. – Tegoni and co-workers[73] have shown that cytochrome *c* can be diffused into crystals of flavocytochrome b_2 where both cytochromes can be oxidized by ferricyanide. Both cytochromes are also reduced by L-lactate, and since the catalytic action of flavocytochrome b_2 is required for the reduction of cytochrome *c* a catalytically competent complex must have been formed.

Cytochrome *c* Complexes. – The preferred sites for electron transfer between cytochrome *c* and iron and cobalt complexes have been examined,[74] and the introduction of cytochrome *c* with azurin and plastocyanin has been studied.[75]

5 Lysozymes and Ribonucleases

Lysozymes. – Blake and co-workers[76] have studied the water structure of lysozyme crystals at high resolution. Human and tortoise egg-white lysozymes contain about 350 and 650 water molecules, respectively. In the crystals 60–80% of the water is represented by featureless electron density, and the number of ordered water molecules placed is 140 for human and 128 for tortoise. The ordered water molecules form a single layer around the protein and are hydrogen-bonded to either polar protein groups or other bound water molecules. Comparisons are also given of the locations of the water molecules on the two lysozyme molecules and show that the multiply bound water molecules occupy similar binding sites. This suggests that crystal packing or the presence of salt ions does not have a dominating effect on the protein–water interactions and that these interactions may therefore correspond to those in solution.

Hydrogen exchange in hen egg-white lysozyme has been examined in solution by n.m.r. and in the crystalline state by neutron diffraction.[77] The results suggest that the average structures in the two states are closely similar and the fluctuations about these two states are also very alike.

The structure of Embden goose lysozyme has been determined at 0.21 nm resolution[78] and the model of the molecule compared with hen egg-white and phage T4 lysozymes. The goose lysozyme has striking similarities in structure to the hen and phage enzymes despite the fact that there is no homology in sequence. Certain areas are common to all three enzymes whereas other areas are common only to pairs of structures. The nature of the correspondences seen suggests that all three lysozymes evolved from a common precursor.

[73] M. Tegoni, A. Mozzarelli, G. L. Rossi, and F. Labeyrie, *J. Biol. Chem.*, 1983, **258**, 5424.
[74] J. Butler, S. K. Chapman, D. M. Davies, A. G. Sykes, S. H. Speck, N. Osheroff, and E. Margoliash, *J. Biol. Chem.*, 1983, **258**, 6400.
[75] M. A. Augustin, S. K. Chapman, D. M. Davies, A. G. Sykes, S. H. Speck, and E. Margoliash, *J. Biol. Chem.*, 1983, **258**, 6405.
[76] C. C. F. Blake, W. C. A. Pulford, and P. J. Artymiuk, *J. Mol. Biol.*, 1983, **167**, 693.
[77] G. A. Bentley, M. Delepierre, C. M. Dobson, R. E. Wedin, S. A. Mason, and F. M. Poulsen, *J. Mol. Biol.*, 1983, **170**, 243.
[78] M. G. Grütter, L. H. Weaver, and B. W. Matthews, *Nature (London)*, 1983, **303**, 828.

Bovine Pancreatic Ribonuclease. – The structure of ribonuclease A has been refined[79] using *X*-ray and neutron data extending to 0.2 nm resolution. The final model contains all the expected protein atoms, 128 water molecules, and a phosphate ion in the active site and gives *R*-factors of 0.159 and 0.183 for the *X*-ray and neutron refinements, respectively. Several side chains have had to be relocated, including the active-site lysine-41, which is now seen to salt-bridge to the phosphate ion. Large modifications to the model of the bound solvent have also had to be made. It has also been possible, using the neutron data only, to show that about 25% of the amide hydrogens are protected against exchange even after one year's exposure to D_2O.

The active-site conformation and specificity of ribonuclease A have been examined using binding studies of inhibitors.[80,81] The binding of 8-oxo-guanosine 2′-phosphate occurs at the pyrimidine-binding site, expelling water molecules from the cleft.[80] The active-site-residue side chains of Gln-11, His-12, and Thr-45 do not alter on inhibitor binding. The side chain of Lys-41 shows reduced mobility and it interacts directly with the 2′-phosphate. The inhibitor binds in the *syn* conformation with the Oγ and NH of Thr-45 involved in base recognition.[81] Side chains close to the catalytic cleft allow for flexibility in substrate binding by their mobility, and inhibitor binding is seen to alter solvent structure of the protein without altering the enzyme's overall shape.

Kuwajima and Baldwin have determined by n.m.r. the locations of the most slowly exchanging peptide NH proteins in residues 1–19 of ribonuclease S,[82] and they have also looked in particular at the behaviour of the amide protons in the helix, residues 3–13.[83]

***Bacillus intermedius* Ribonuclease.** – Pavlovsky and co-workers[84] have reported the structure of ribonuclease from *Bacillus intermedius* 7 P. The enzyme is a guanyl-specific ribonuclease with a chain length of 109 residues and is unusual in that it includes no sulphur-containing amino acids. The electron-density map was improved by averaging the density for the two molecules in the asymmetric unit, and the chain can be traced unequivocally. The molecule contains two short helices and a twisted β-sheet of five antiparallel strands and shows great similarity in structure to the *Bacillus amyloliquefaciens* in all areas. Active-site residues have been identified and include the histidine residue His-101 implicated in catalysis by solution studies.

6 Proteolytic Enzymes

Trypsin. – The active-site geometries of 16 protein crystal structures involving trypsin, trypsinogen, or their inhibitors have been analysed.[85] It was found that

[79] A. Wlodawer and L. Sjölin, *Biochemistry*, 1983, **22**, 2720.
[80] N. Borkakoti, *Eur. J. Biochem.*, 1983, **132**, 89.
[81] N. Borkakoti, R. A. Palmer, I. Haneef, and D. S. Moss, *J. Mol. Biol.*, 1983, **169**, 743.
[82] K. Kuwajima and R. L. Baldwin, *J. Mol. Biol.*, 1983, **169**, 281.
[83] K. Kuwajima and R. L. Baldwin, *J. Mol. Biol.*, 1983, **169**, 299.
[84] A. G. Pavolvsky, A. A. Vagin, B. K. Vainstein, N. K. Chepurnova, and M. Ya. Karpeisky, *FEBS Lett.*, 1983, **162**, 167.
[85] M. Marquart, J. Walter, J. Deisenhofer, W. Bode, and R. Huber, *Acta Crystallogr., Sect. B*, 1983, **39**, 480.

the conformation of the active-site triplet Asp-102–His-57–Ser-195 is well conserved irrespective of the different functional states of the enzyme. Refinement of the crystal structures results in improvements in bond lengths and angles of the His-57–Ser-195 hydrogen bond. The inhibitor binding at the main chain and side chain of P1 is seen to be well conserved, and this includes solvent molecules involved in the hydrogen-bond network. Analysis of the peptide groups shows that they may be substantially non-planar.

Trypsin Inhibitor. – Basic pancreatic trypsin inhibitor (PTI) has been crystallized in a new crystal form, and its structure has been determined at 0.094 nm resolution.[86]

Yoshioki and co-workers[87] have described a method for conformational-energy minimization using bovine PTI as an example, and Goldenberg and Creighton[88] have described a circular and a circularly permuted form of bovine PTI.

Kallikrein. – Porcine pancreatic kallikrein A, a serine proteinase that has the ability to release kinins from α2-globulin structures, has been crystallized in the presence of the inhibitor benzamidine in a form with two molecules in the asymmetric unit.[89] The structure has been solved by multiple isomorphous replacement at 0.205 nm resolution and refined to a crystallographic residual, R_f, of 0.220. The two molecules are related by a non-integral screw axis and form open, heterologous 'dimer' structures. Significant differences in conformation between the two molecules occur, especially at surface loops. One active site is occluded by residues from the other molecule in the asymmetric unit whereas the other active site is accessible to the solvent. The cleavage loop, or 'kallikrein loop', is only partially traceable, and the carbohydrate attached in this area is not seen in the model. Comparison with trypsin shows spatial homology, although there are large deviations seen for some external loops that limit the accessibility to the kallikrein active-site cleft. Structural explanation is advanced for the geometry and strength of benzamidine binding.

Kallikrein complexed with bovine PTI has also been crystallized and its structure solved at 0.25 nm resolution.[90] The overall binding of the PTI is very similar to that of the trypsin–PTI complex, and the active site is identical within experimental error. The paper details the specific interactions between the kallikrein molecule and PTI, and from these observations a qualitative explanation for the unusual binding and cleavage at the N-terminal Met-Lys site on kininogen can be deduced. Steric hindrance of protein-inhibitor binding by surface loops around the kallikrein active site may explain kallikrein's restricted binding ability.

[86] J. Walter and R. Huber, *J. Mol. Biol.*, 1983, **167**, 911.
[87] S. Yoshioki, H. Abe, T. Noguti, N. Go, and K. Nagayama, *J. Mol. Biol.*, 1983, **170**, 1031.
[88] D. P. Goldenberg and T. E. Creighton, *J. Mol. Biol.*, 1983, **165**, 407.
[89] W. Bode, Z. Chen, K. Bartels, C. Kutzbach, G. Schmidt-Kastner, and H. Bartunik, *J. Mol. Biol.*, 1983, **164**, 237.
[90] Z. Chen and W. Bode, *J. Mol. Biol.*, 1983, **164**, 283.

Carboxypeptidase A. – A refined crystal structure of carboxypeptidase A at 0.154 nm resolution has been reported.[91] The final model gives an *R*-factor of 0.190 and shows four examples of a reverse turn, which requires an aspartate or asparagine as one of the residues. In this Asx turn the turn-closing hydrogen bond is between the Asx side-chain C=O group and a peptide NH. A total of 192 water molecules have been located in the structure, and analysis of the CO···N and NH···O hydrogen-bond angles in α-helices and β-sheets shows that they centre at about 156°. The CO...O(water) hydrogen-bond angle is, in contrast, distributed about 131°, indicating the role of the lone-pair electrons of the carbonyl oxygen in the hydrogen bond. Of the 192 water molecules 24 are buried in the protein and several of these are organized into hydrogen-bonded chains containing up to five water molecules.

A cryoenzymological study of a true reaction intermediate of carboxypeptidase A has allowed a proposal of the true catalytic conformation of the enzyme to be proposed.[92]

Thermolysin. – The binding of an active-site-directed irreversible inhibitor to thermolysin has been studied by *X*-ray diffraction at 0.23 nm resolution.[93] The inhibitor, an *N*-hydroxy derivative of double-blocked leucine, alkylates Glu-143 in the thermolysin active site, and the hydroxamic acid group co-ordinates the zinc ion. Binding of the inhibitor leads to a significant shift in a segment of the polypeptide chain near the active site, and alkylation would be prevented if this shift did not occur. Implications for the catalytic functioning of the enzyme towards true substrates are presented.

***Streptomyces griseus* Protease B.** – The 0.18 nm resolution structure of *Streptomyces griseus* protease B molecule complexed with the third domain of the Kazal-type ovomucoid from turkey has been determined[94] and refined to an *R*-factor of 0.125. The inhibitor binds in a manner similar to a substrate but is not cleaved, although there is a close approach of the Oδ atom of Ser-195 to the carbonyl carbon of the inhibitor's reactive peptide bond. In direct contrast to other reports the authors conclude that this peptide bond does not deviate from planarity. Inhibition can be understood in terms of the equilibrium thermodynamic parameters, which can be rationalized in terms of the observed structure.

Penicillopepsin. – The refinement of the 0.18 nm structure of the aspartyl protease penicillopepsin has been reported.[95] The average co-ordinate error is less than 0.012 nm, and the final *R*-factor is 0.136. The structure shows a relatively low average temperature factor, partly because of the large central hydrophobic core, in which there are two prominent clusters of aromatic residues. The N- and C-terminal domains are related by approximate two-fold symmetry, related by a rotation of 177° and a translation of 0.12 nm. The authors present

[91] D. C. Rees. M. Lewis, and W. N. Lipscomb, *J. Mol. Biol.*, 1983, **168**, 367.
[92] L. C. Kuo, J. M. Fukuyama, and M. W. Makinen, *J. Mol. Biol.*, 1983, **163**, 63.
[93] M. A. Holmes, D. E. Tronrud, and B. W. Matthews, *Biochemistry*, 1983, **22**, 236.
[94] R. J. Read, M. Fujinaga, A. R. Sielecki, and M. N. G. James, *Biochemistry*, 1983, **22**, 4420.
[95] M. N. G. James and A. R. Sielecki, *J. Mol. Biol.*, 1983, **163**, 299.

an analysis of the hydrogen-bond geometries in the various secondary structures and have also determined the side-chain torsional-angle distributions.

There are several solvent molecules in the active site, one of which is hydrogen-bonded to the two active-site aspartate residues. The positions of the two aspartates suggest that neither is likely to be involved in a direct nucleophilic attack on the scissile bond of the substrate, and temperature factor analysis indicates that conformational mobility in the active site has no role in the mechanism. Other more mobile regions have been identified in this way.

The positions of 319 solvent sites have been identified and refined, 13 of which are internal and several of which are possibly catalytically important. Intermolecular contacts involve many residues and often also have bridging water molecules positioned between polar groups on the surface of the protein molecules.

Renin. – Blundell and co-workers[96] have proposed a model for the structure of renin based on the known crystal structure of another aspartyl protease, Endothia pepsin. A structure for renin that is similar to that of the other aspartyl proteases can be proposed.

7 Glycolytic and Tricarboxylic Acid Cycle Enzymes

Glycogen Phosphorylase. – The binding sites for the allosteric activator AMP on glycogen phosphorylase *b* have been described in detail.[97] The improved detail has been achieved using a more accurate model for the native enzyme. The effect of AMP binding involves localized conformational changes of 0.1–0.2 nm for Tyr-75 and Arg-309 and small shifts for residues 42–44 on the symmetry-related subunit. Binding studies have also shown that NADH binds at the allosteric effector site N, close to the subunit–subunit interface and the nucleoside inhibitor site I, which is some 1.2 nm from the catalytic site. The NADH conformations in the two sites are different but in both cases the molecule is folded with the nicotinamide and adenine rings only 0.6 nm apart.

Oligosaccharide binding to phosphorylase *b* has been studied by difference Fourier analysis.[98] Four different oligosaccharides containing different numbers of glucose residues have all been shown to bind at the glycogen storage site on the surface of the enzyme. No binding was seen at the catalytic site. Eight binding sites that are occupied with different affinities by the various oligosaccharides have been identified, and the major contacts with the enzyme involve the long α-helix. In some experiments binding of substrates or effectors at the catalytic site weakens binding of the oligosaccharide at the storage site and *vice versa*. It may even be possible for a long polysaccharide to wrap itself around the outside of the enzyme and for the non-reducing end to bind at the catalytic site.

[96] T. Blundell, B. L. Sibanda, and L. Pearl, *Nature (London)*, 1983, **304**, 273.
[97] E. A. Stura, G. Zanotti, Y. S. Babu, M. S. P. Sansom, D. I. Stuart, K. S. Wilson, L. N. Johnson, and G. Van de Werve, *J. Mol. Biol.*, 1983, **170**, 529.
[98] L. N. Johnson, E. A. Stura, M. S. P. Sansom, and Y. S. Babu, *Biochem. Soc. Trans.*, 1983, **11**, 142.

6-Phosphogluconate Dehydrogenase. – Adams and co-workers[99] have described an improved model of 6-phosphogluconate dehydrogenase based on a new electron-density map. Further detail has become apparent in the small domain, and several chain connections have had to be altered. The coenzyme binds to the small domain, which contains a β-α-β structure, with the adenine at the enzyme surface at the edge of the β-sheet and the rest of the NADP bound in the cleft between the two domains. The nicotinamide group binds deepest in the cleft and points towards the large domain. The authors also discuss structure–function relationships of NAD^+- and $NADP^+$-dependent dehydrogenases, and they suggest that the minimum requirement for the most frequent binding mode of the coenzymes is a sheet with a twist in it and a helix on either side.

Glyceraldehyde-3-phosphate Dehydrogenase. – Leslie and Wonacott[100] have described the crystallization of apoglyceraldehyde-3-phosphate dehydrogenase from *Bacillus stearothermophilus*. Partially NAD^+-saturated enzyme crystallizes isomorphously with the apoenzyme, and difference maps show that for crystals containing one NAD^+ molecule per tetramer it is uniquely bound in one of the four available sites. This addition of NAD^+ gives rise to non-equivalence in the binding of a heavy-atom compound whereas for the apoenzyme the binding is symmetric. These results give strong evidence that the binding of NAD^+ gives rise to sequential ligand-induced conformational changes in the tetramer. These changes may be responsible for the observed negative co-operativity in coenzyme binding.

The glyceraldehyde-3-phosphate dehydrogenase from the thermophile *Bacillus coagulans* has been crystallized[101] and the molecule shown to have an exact two-fold rotation axis in one of the crystal forms. Using a variety of crystallographic techniques this axis has been identified as the molecular *R*-axis, the axis relating to two subunits that form each active site of the tetrameric enzyme. This is in contrast to the human enzyme where the crystallographically exact axis was found to be the *Q*-axis. These results could have important implications for the allosteric behaviour of the enzyme.

Alcohol Dehydrogenase. – Plapp and co-workers[102] have crystallized liver alcohol dehydrogenase that has been modified by amidination. The modified enzyme, treated with methyl isonicotinimidate, shows a ten-fold increase in activity, and the *X*-ray results show that 23 of the 30 lysine residues are derivatized, two are buried, and five are accessible but not substituted. The overall conformation of the modified enzyme is very similar to that of the native enzyme despite extensive modification, and only a few regions show movements of 0.1–0.2 nm for α-carbon atoms. When NADH binds, the isonicotinimidyl group on lysine-228 interferes with the normal interactions that occur between the lysine side

[99] M. J. Adams, S. Gover, R. W. Pickersgill, and J. R. Helliwell, *Biochem Soc. Trans.*, 1983, **11**, 429.

[100] A. G. W. Leslie and A. J. Wonacott, *J. Mol. Biol.*, 1983, **165**, 375.

[101] J. P. Griffith, B. Lee, A. L. Murdock, and R. E. Amelunxen, *J. Mol. Biol.*, 1983, **169**, 963.

[102] B. V. Plapp, H. Eklund, T. A. Jones, and C.-I. Brändén, *J. Biol. Chem.*, 1983, **258**, 5537.

chain and the ribophosphate of the AMP part of the coenzyme. The enzyme is therefore activated because coenzyme dissociates more rapidly.

The crystal structure of horse-liver alcohol dehydrogenase in a complex with NADH and imidazole has been determined at 0.24 nm resolution.[103] The enzyme complex crystallizes in the apo conformation in which the active-site cleft is not totally shielded from the solvent. The NADH binds in an extended conformation, and the enzyme–coenzyme interactions are weaker when compared to other complexes. The carboxamide group of the nicotinamide ring lies within hydrogen-bonding distance of the sulphur of Cys-46, one of the three protein ligands to the active-site zinc atom. The imidazole molecule is directly ligated to the zinc atom, which shows roughly tetrahedral geometry in this complex.

Malate Dehydrogenase. – The structure of cytoplasmic malate dehydrogenase has been refined by crystallographic least-squares methods[104] and has led to a new model of the structure being proposed. A new tentative amino acid sequence has been derived, and the two independent subunits in the asymmetric unit are shown to be nearly identical and related by an approximate two-fold axis. The active site contains a histidine-aspartate pair, hydrogen-bonded to each other, which may act as a proton-relay system, and a similar pairing exists in lactate dehydrogenase. The authors also describe how the active-site stereochemistry for these two enzymes can explain the stronger binding of NADH compared with NAD^+ binding.

Roderick and Banaszak[105] have described the 0.53 nm structure of mitochondrial malate dehydrogenase. The results show that the mitochondrial and cytoplasmic enzymes are very similar, and the locations of the nucleotide-binding sites have been determined from difference electron-density maps.

8 Hormones

Insulin. – A review of the current state of knowledge of insulin structure has been published,[106] and structure–function studies have been discussed.[107] Some of the problems encountered in studying the structures of insulin derivatives have been reported.[108]

Molecular-replacement studies on modified insulins have been published.[109] Bovine and sheep insulin, modified by the removal of the five C-terminal residues from the B-chain, have been crystallized in several crystal forms, and *X*-ray data have been collected to 0.21 nm resolution. These data were then analysed by the fast-rotation function and *R*-factor searches, allowing reasonably good models to

[103] E. Cedergren-Zeppezauer, *Biochemistry*, 1983, **22**, 5761.
[104] J. J. Birktoft and L. J. Banaszak, *J. Biol. Chem.*, 1983, **258**, 472.
[105] S. L. Roderick and L. J. Banaszak, *J. Biol. Chem.*, 1983, **258**, 11 636.
[106] D. C. Hodgkin, E. Dodson, G. Dodson, and C. Reynolds, *Biochem. Soc. Trans.*, 1983, **11**, 411.
[107] Y.-S. Zhang, *Biochem. Soc. Trans.*, 1983, **11**, 417.
[108] L. Dong-Cai, D. Jin-Bi, D. Stuart, W. Zhu-Li, R. Todd, Y. Jun-Ming, and L. Mei-Zhen, *Biochem. Soc. Trans.*, 1983, **11**, 419.
[109] B. Ru-Chang, S. M. Cutfield, E. J. Dodson, G. G. Dodson, F. Giordano, C. D. Reynolds, and S. P. Tolley, *Acta Crystallogr., Sect. B*, 1983, **39**, 90.

be derived. The packing of the modified sheep insulin in the C2 crystal form is quite different from all other insulins studied so far, even though the molecular structure is similar to that of 2-Zn pig insulin.

The amino acid sequence of dogfish insulin has been determined,[110] and computer-graphics model building has predicted that the dogfish insulin molecule can attain a structure very similar to that of bovine insulin.

Chothia and co-workers[111] have discussed the transmission of conformational change in insulin. The slight conformational changes in insulin molecules occur with packed helices moving relative to each other. These helix shifts are accommodated by small movements of side chains, and since these movements can accommodate shifts of no more than 0.15 nm the shifts become translated over long distances.

9 Other Globular Proteins

Alkaline Phosphatase. – Three metal-ion sites have been located[112] in each of two symmetric active centres of alkaline phosphatase from *E. coli*. These two active centres are 3.2 nm apart, and a product analogue, arsenate, is found to bind adjacent to the active-centre serine and close to two of the metal-ion sites; an arginine-residue side chain also lies close to the arsenate. It seems that the arginine and two metal ions will neutralize the charge on the phosphate, activate the serine hydroxyl group, and stabilize the transition state. Strong antagonism is seen between binding at these two ion sites but is relieved by phosphate binding.

L-Arabinose-binding Protein. – Mao and McCammon[113] have described a theoretical study of the hinge-bending suggested for L-arabinose-binding protein. The results show that the unliganded protein is stabilized in an open conformation by the exposure and solvation of charged groups in the cleft. Ligand binding induces the closure of the cleft.

Aspartate Aminotransferase. – Kirsten and co-workers[114] have described how in the crystalline state aspartate aminotransferase shows asymmetry of substrate binding. One subunit binds aspartate and 2-oxoglutarate with Km's of 0.5 mM and 1.2 mM, respectively, whereas the other subunit shows Km's of 5.5 mM and 14.5 mM. Since the two subunits are apparently identical this functional asymmetry is most probably the result of the conformational flexibility of the enzyme being restrained by crystal-lattice forces.

[110] M. Bajaj, T. L. Blundell, J. E. Pitts, S. P. Wood, M. A. Tatnell, S. Falkmer, S. O. Emdin, L. K. Gowan, H. Crow, C. Schwabe, A. Wollmer, and W. Strassburger, *Eur. J. Biochem.*, 1983, **135**, 535.

[111] C. Chothia, A. M. Lesk, G. G. Dodson, and D. C. Hodgkin, *Nature (London)*, 1983, **302**, 500.

[112] J. M. Sowadski, M. D. Handschumacher, H. M. K. Murthy, C. E. Kundrot, and H. W. Wyckoff, *J. Mol. Biol.*, 1983, **170**, 575.

[113] B. Mao and J. A. McCammon, *J. Biol. Chem.*, 1983, **258**, 12 543.

[114] H. Kirsten, H. Gehring, and P. Christen, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 1807.

ATPases. – The 0.9 nm resolution structure of the F1 sector of ATPase has been described.[115] The molecule is seen to have two-fold symmetry that is inexplicable in terms of the previously accepted stoicheiometry of $\alpha_3\beta_3\gamma\delta\epsilon$. The molecule shows six masses of density arranged as a flattened octahedron with a distinct cavity at the centre of the molecule. Various models are proposed and examined with reference to known binding of inhibitors and antibiotics.

Bacteriochlorophyll A Protein. – Soman and Ramakrishnan[116] have reported the occurrence of an eight-residue long segment of collagen helical structure at residues 277–284 of bacteriochlorophyll A protein. The structure has been identified using a secondary-structure-determining algorithm and is the longest stretch of collagen helix found in globular proteins.

***cro* Repressor.** – Comparison of *cro* repressor with the amino-terminal domain of λ-repressor protein, both from bacteriophage λ, has shown that the second and third helices have identical conformations.[117] Parts of the first helix also show a structural correspondence that leads to a revision of the sequence homology at the extreme N-terminal region. In particular, a short loop between the first and second helices is deleted from the *cro* structure and may explain known chemical properties of the proteins. Although the α_2–α_3 units are very similar, stereochemical constraints prevent these units from aligning on DNA in exactly the same way.

γ-II Crystallin. – The structure analysis and refinement of calf γ-II crystallin have been reported at 0.19 nm resolutions.[118] The protein has a symmetrical structure of two domains, each comprising two similar 'Greek key' motifs and related by a pseudo-two-fold axis. In each domain the two Greek key motifs form a pair of four-stranded antiparallel β-pleated sheets packed together as a wedge shape. In the core of each domain there are interactions between polarizable aromatic groups and sulphur-containing residues. This not only confers stability but also protects the aromatic side chains from u.v. light damage in the lens. On the surface over half the ionic side chains are closely paired, which increases stability and decreases the amount of water bound. Possible structural changes involved in cataract formation are also discussed and seem to involve the formation of intermolecular disulphide bridges.

DNA Polymerase I. – A 0.7 nm resolution structure determination of the large fragment of *E. coli* DNA polymerase I has been reported.[119] The protein consists of two domains and is seen to be mostly composed of α-helices. The active site has been located by binding several nucleoside tri- and mono-phosphates and lies in a pocket on the surface of the molecule.

[115] L. M. Amzel, P. Narayanan, P. L. Pedersen, and J. Sygusch, *Ann. N.Y. Acad. Sci.*, 1982, **402**, 21.
[116] K. V. Soman and C. Ramakrishnan, *J. Mol. Biol.*, 1983, **170**, 1045.
[117] D. H. Ohlendorf, W. F. Anderson, M. Lewis, C. O. Pabo, and B. W. Matthews, *J. Mol. Biol.*, 1983, **169**, 757.
[118] G. Wistow, B. Turnell, L. Summers, C. Slingsby, D. Moss, L. Miller, P. Lindley, and T. Blundell, *J. Mol. Biol.*, 1983, **170**, 175.
[119] P. Brick, D. Ollis, and T. A. Steitz, *J. Mol. Biol.*, 1983, **166**, 453.

D-Galactose-binding Protein. – The 0.3 nm resolution structure of *E. coli* D-galactose-binding protein has been reported.[120] The structure was determined by molecular replacement at 0.32 nm resolution and by multiple isomorphous replacement at 0.3 nm resolution. The overall shape of the molecule is closely similar to that of L-arabinose-binding protein, being composed of two globular domains. The two lobes contain a core of β-pleated sheet with a pair of helices on either side of the sheet, and the four hydrophobic clusters that maintain the L-arabinose-binding protein structure are also present in the D-galactose-binding protein molecule.

The *X*-ray structure of galactose-binding protein from *Salmonella typhimurium* has been determined at 0.3 nm resolution.[121] The molecule is a prolate ellipsoid, with dimensions 3.5 nm × 3.5 nm × 6.5 nm, and consists of two roughly equally sized domains separated by a 0.8 nm wide cleft. Each domain is composed of a core of parallel β-pleated sheet surrounded by five helices and shows a very similar folding to that of L-arabinose-binding protein and D-galactose-binding protein from *E. coli*. Two areas on the surface of the long side of the molecule do differ, and these differences are adequate to explain the different interactions of L-arabinose-binding protein and D-galactose-binding protein with the membrane proteins involved in transport and chemotaxis.

Gene 5 DNA-binding Protein. – The refined structure of the gene 5 DNA-binding protein (G5BP) from bacteriophage fd has been determined at 0.23 nm resolution.[122] The monomer of 87 residues is almost entirely composed of β-structure organized as a three-stranded sheet, a two-stranded β-ribbon, and a broad connecting loop. Two G5BP molecules are tightly interlocked about a two-fold axis to form a dimer characterized by two symmetry-related antiparallel clefts that traverse the surface. From the three-stranded antiparallel β-sheet extend three tyrosine residues, a lysine and two arginine residues that are involved in DNA binding. From the other monomer in the dimer an arginine and a phenylalanine from the β-ribbon are also involved in the binding of DNA, meaning that the DNA-binding area is formed by the two subunits. The G5BP dimer is the active species with the two monomers so closely interwoven that it seems unlikely for the monomer to maintain this observed structure in solution. From each monomer extends an elongated wing, which comprises residues 15–32 and is mobile and seems likely to be involved in DNA binding.

Glutaminase-asparaginase. – Ammon and co-workers[123] have described a rotation-function study of glutaminase-asparaginase from *Pseudomonas* 7A and *Acinetobacter*. The *Acinetobacter* enzyme has one subunit in the asymmetric unit with the subunits arranged with 222 symmetry. The *Pseudomonas* enzyme has a tetramer in the asymmetric unit, and the rotation function shows that the subunits of this enzyme are also arranged with 222 symmetry.

[120] N. K. Vyas, M. N. Vyas, and F. A. Quiocho, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 1792.
[121] S. L. Mowbray and G. A. Petsko, *J. Biol. Chem.*, 1983, **258**, 7991.
[122] G. D. Brayer and A. McPherson, *J. Mol. Biol.*, 1983, **169**, 565.
[123] H. L. Ammon, K. C. Murphy, L. Sjölin, A. Wlodawer, J. S. Holcenberg, and J. Roberts, *Acta. Crystallogr., Sect. B*, 1983, **39**, 250.

Glutathione Peroxidase. – The refined structure of glutathione peroxidase at 0.2 nm resolution has been described,[124] and the agreement of the two independently refined monomers is very close. A total of 165 water molecules have been located, and the hydrogen-bonding pattern and mobility of the structure have been analysed. The active centres are located at flat-surface depressions, and the catalytically active selenocysteine residues could be located at the N-terminal region of helices in β–α–β units. Several other active-site residues, including a tryptophan (Trp-148), have been identified. Substrate- and inhibitor-binding studies, in the crystal and in solution, can be interpreted by an apparent half-of-the-sites reactivity, and a model of glutathione binding has been derived.

Glutathione Reductase. – The two nucleotide-binding domains of glutathione reductase have been compared with the FAD-binding fold of *p*-hydroxybenzoate hydroxylase.[125] The fold, which consists of a four-stranded parallel β-sheet sandwiched between a three-stranded antiparallel sheet and α-helices, binds nucleotides in similar relative positions. The best superposition gives 110 equivalent residue positions of which only four are identical in all three domains. In both enzymes the *si* face of the FAD isoalloxazine is covered by polypeptide and only the *re* face is accessible to NADPH. In each enzyme there is a long helix that points with its N-terminal part at the 0–2α region of the isoalloxazine ring, and this interaction may be catalytically important in neutralizing the negative charge at 0–2α.

A topography for leucocyte NADPH oxidase, a membrane flavoenzyme, has been suggested based on the glutathione reductase structure.[126]

Pai and Schulz[127] have determined the binding mode of NADPH and oxidized glutathione to glutathione reductase at 0.3 nm resolution. Two reaction intermediates have also been produced and their structures determined. The NADPH binds in an extended conformation with the nicotinamide ring stacking on the *re* face of the FAD isoalloxazine ring and the adenine at the molecular surface. The binding of NADPH results in a two-electron form of the enzyme EH_2, which has also been analysed in the absence of ligand. In EH_2 the redox-active disulphide, which lies at the *si* face of the flavin ring, opens, and one of the sulphurs moves to where it can attack one of the sulphurs of oxidized glutathione. This mixed glutathione–protein disulphide can be stabilized in crystals and has been analysed. The cysteine can also be blocked by reaction with iodoacetate and blocked in the EH_2 state. A histidine side chain lies near all the sulphurs taking part in the disulphide-bridge exchange and is crucial in catalysis. The results show that electrons flow from NADPH to oxidized glutathione *via* the flavin rings and the redox-active disulphide bridge. This scheme agrees with that proposed from other studies.

Histidine Decarboxylase. – Several crystal forms of histidine decarboxylase (HDC) and prohistidine decarboxylase (PHDC) have been obtained; all contain

[124] O. Epp, R. Ladenstein, and A. Wendel, *Eur. J. Biochem.*, 1983, **133**, 51.
[125] R. K. Wierenga, J. Drenth, and G. F. Schulz, *J. Mol. Biol.*, 1983, **167**, 725.
[126] R. H. Schirmer, G. E. Schulz, and R. Untucht-Grau, *FEBS Lett.*, 1983, **154**, 1.
[127] E. F. Pai and G. E. Schulz, *J. Biol. Chem.*, 1983, **258**, 1752.

multiple protomers in the asymmetric unit.[128] Data have been collected to 0.56 nm resolution for HDC and 0.55 nm for PHDC. Rotation-function studies show that both proteins possess 32 molecular symmetry and the molecules have been related to each other in their respective unit cells.

L-3-Hydroxyacyl CoA Dehydrogenase. – The structure of L-3-hydroxyacyl CoA dehydrogenase (L3HCD) has been determined at 0.525 nm resolution.[129] In the L3HCD crystal form the asymmetric unit contains 1.5 dimers of the molecule, one dimer lying at a general position, the other on a two-fold axis. Each subunit shows a bilobal structure; the larger lobe, which binds NAD^+, is about 3.7 nm × 4.5 nm × 3.5 nm, and the smaller lobe is 3.0 nm × 2.3 nm × 2.0 nm. From difference Fourier maps it is seen that NAD^+ binds to the L3HCD molecule in an open conformation.

***p*-Hydroxybenzoate Hydroxylase.** – The primary sequence of *p*-hydroxybenzoate hydroxylase has been determined and fitted to a 0.25 nm resolution electron-density map.[130] The residues contributing to FAD and substrate binding have been identified. The FAD binds in an extended conformation and is tightly bound by hydrogen bonding, electrostatic interaction, and hydrophobic packing at an active site in a large hydrophobic area enclosed by all domains of the enzyme structure. The substrate, *p*-hydroxybenzoate, is bound approximately perpendicular to the flavin moiety and is completely buried in the interior of the molecule. The residues involved in subunit–subunit interactions have been identified, and a largely hydrophobic intersubunit contact area has been located.

Leucine-, Isoleucine-, and Valine-binding Protein. – The 0.3 nm crystal structure of leucine-, isoleucine-, and valine-binding protein (LIVBP) from *E. coli* has been reported.[131] The LIVBP molecule is composed of two globular domains connected by three strands, each domain being composed of a β-sheet core flanked by at least two helices on either side. Difference Fourier maps have located a single amino acid-binding site for L-leucine, L-isoleucine, and L-valine. This site is located in the cleft between the two domains. Despite a lack of sequence homology the overall molecular conformation is very similar to that found in L-arabinose- and D-galactose-binding proteins.

Monellin. – The 0.55 nm resolution structure of monellin has been reported.[132] The protein is 10^5 times sweeter than sucrose when compared on a molar basis and is composed of two non-identical protein chains with a total molecular weight of 10 700. The low-resolution structure does reveal many protruding features, and it may be that these protrusions interact with the sweet receptor.

Neurotoxin from Scorpion. – Variant-3 scorpion neurotoxin has been crystallized and its structure determined at 0.18 nm resolution.[133] The refined model includes

[128] E. H. Parks, K. Clinger, and M. L. Hackert, *Acta Crystallogr., Sect. B*, 1983, **39**, 490.
[129] H. M. Holden and L. J. Banaszak, *J. Biol. Chem.*, 1983, **258**, 2383.
[130] W. J. Weijer, J. Hofsteenga, J. J. Beintema, R. K. Wierenga, and J. Drenth, *Eur. J. Biochem.*, 1983, **133**, 109.
[131] M. A. Saper and F. A. Quiocho, *J. Biol. Chem.*, 1983, **258**, 11 057.
[132] G. Tomlinson, C. Ogata, W.-C. Shin, and S.-H. Kim, *Biochemistry*, 1983, **22**, 5772.
[133] F. J. Almassy, J. C. Fontecilla-Camps, F. L. Suddath, and C. E. Bugg, *J. Mol. Biol.*, 1983, **170**, 497.

492 protein atoms from the 65 residues, one molecule of 2-methylpentane-2,4-diol, and 72 solvent sites. The structure contains two and a half turns of α-helix and a three-strand stretch of antiparallel β-sheet, the helix being connected to the central β-sheet strand by two disulphide bridges. Several loops extend from this compact central structure and display several reverse turns and a proline-rich C-terminus. One proline residue adopts a *cis*-peptide conformation. The molecule also shows a large flattened surface containing a high proportion of hydrophobic residues together with most of the conserved residues found in scorpion neurotoxins.

Phospholipase A2. – A redetermination of the structure of porcine pancreatic phospholipase A2 has been published.[134] Previous work had suggested that, despite very homologous amino acid sequences, the structures of the pig and beef enzymes were not similar. The redetermination of the pig enzyme involved molecular replacement using the beef enzyme as the parent, and the crystals were found to be of phospholipase A2, the prophospholipase A2 having been slowly converted under the crystallizing conditions employed. The two molecules are now seen to be very similar, and this includes the active site. In one loop, 59–70, where a single amino acid substitution occurs (bovine Val-63–porcine Phe), a complete rearrangement of the peptide chain occurs. In addition to the active-centre calcium ion a second calcium is found located on the two-fold axis, where it stabilizes intersubunit interactions.

Rhodanese. – The binding of four different metal cyanides to bovine-liver rhodanese has been studied by difference Fourier analysis.[135] These compounds, all inhibitors of the enzyme, bind at a common position at the entrance to the active-site pocket, where they interact with a lysine and an arginine residue. One of the complexes, sodium aurocyanide, also binds at three cysteine residues, at one of which, Cys-247, it displaces the extra sulphur atom in the sulphur–rhodanese complex. The zinc cyanide complex binds to totally different additional sites and forms complexes with an aspartate and a histidine residue. Chemical explanations for these different binding modes are advanced.

10 tRNA Synthetase/tRNA

tRNA Synthetase. – The structures of methionyl-tRNA synthetase from *E. coli* and tyrosyl-rRNA synthetase from *Bacillus stearothermophilus* have been compared[136] and show close similarity over a span of about 140 amino acids. The homologous region corresponds to a five-stranded parallel β-sheet, including a mononucleotide-binding fold. A cysteine and two histidines found to be invariant in the amino acid sequences occupy equivalent positions in the nucleotide-binding fold. In tyrosyl-tRNA synthetase these residues are close to the

[134] B. W. Dijkstra, R. Renetseder, K. H. Kalk, W. G. J. Hol, and J. Drenth, *J. Mol. Biol.*, 1983, **168**, 163.

[135] L. J. Lijk, K. H. Kalk, N. P. Brandenburg, and W. G. J. Hol, *Biochemistry*, 1983, **22**, 2952.

[136] D. M. Blow, T. N. Bhat, A. Metcalfe, J. L. Risler, S. Brunie, and C. Zelwer, *J. Mol. Biol.*, 1983, **171**, 571.

adenylate-binding site, while in the methionyl-tRNA synthetase they lie close to the Mg^{2+}–ATP-binding site.

$tRNA^{Phe}$. – An *X*-ray study of a zinc(II) complex of yeast $tRNA^{Phe}$ has shown the presence of five zinc-binding sites in the tRNA molecule.[137] Two of the Mg^{2+} ions in the native tRNA structure are replaced by zinc ions. The other three sites involved co-ordination of zinc to the N-7 position of three guanine bases, suggesting a high specificity for zinc binding to guanine bases in purine-purine sequences.

$tRNA_f^{Met}$. – Sussman and Podjarny[138] have described the use of a constrained–restrained least-squares procedure for refining the structure of yeast $tRNA_f^{Met}$. The method uses large groupings, for example helical domains, as refinement units that are then gradually broken down to smaller units. The $tRNA_f^{Met}$ refinement resolution ranged from 1.25 nm to 0.4 nm resolution, and the final model, which has acceptable stereochemistry, converged to an *R*-factor of 0.265.

11 Viruses

Influenza-virus Neuraminidase. – The structure of the influenza-virus glycoprotein antigen neuraminidase has been determined at 0.29 nm resolution.[139] This enzyme is one of the two glycoproteins embedded in the membrane of the virus and catalyses a reaction that allows transport of the virus through mucin and destroys the haemagglutin receptor on the host cell, thus allowing escape of the progeny virus from the infected cell.

Two different strains have been solved and show very similar structures, the tetramers being a box-shaped head, 10 nm × 10 nm × 6 nm, attached to a slender stalk. Each subunit is composed of six topologically identical β-sheets, each of four strands connected by reverse turns, which form a propeller structure. The positions of the disulphide bridges and the binding sites of the carbohydrate moieties and metal ions have also been located. The enzyme provides the first example of a tetrameric protein with circular four-fold symmetry.

Colman and co-workers[140] have also discussed the location of the catalytic and antigenic sites on the neuraminidase enzyme. The catalytic sites lie at the upper corners of the box-shaped head of the tetramer. Antigenic determinants form an almost continuous surface on the top of the molecule encircling the catalytic site. The numbers and positions of the amino acid changes in these antigenic determinants between 1968 and 1975 have been described and compared with changes in the antigenic sites of the haemagglutin molecule.

Influenza-virus Haemagglutinin. – The structure and role of the carbohydrate moieties attached to the influenza-virus haemagglutin have been reported.[141] In

[137] J. R. Rubin, J. Wang, and M. Sundaralingam, *Biochim. Biophys. Acta*, 1983, **756**, 111.
[138] J. L. Sussman and A. D. Podjarny, *Acta Crystallogr., Sect. B*, 1983, **39**, 495.
[139] J. N. Varghese, W. G. Laver, and P. M. Colman, *Nature (London)*, 1983, **303**, 35.
[140] P. M. Colman, J. N. Varghese, and W. G. Laver, *Nature (London)*, 1983, **303**, 41.
[141] I. A. Wilson, R. C. Ladner, J. J. Skehel, and D. C. Wiley, *Biochem. Soc. Trans.*, 1983, **11**, 145.

the 1968 Hong Kong influenza-virus haemagglutinin seven glycosylated positions have been identified, and 17 of the 64 sugar hexoses have been located in the electron-density map. The role of the carbohydrate may be concerned with masking potential chymotrypsin or trypsin cleavage sites or with antigenic variation by masking areas of the surface that interact with the immune system.

The antigenic variation in the haemagglutinin has also been studied.[142] Four reasonably discrete areas on the surface of the molecule have been identified where point mutations affect the binding of the mutant virus to a monoclonal antibody. The authors also suggest that the location of the carbohydrate has a direct effect on antigenicity.

Single amino acid substitutions in the influenza-virus haemagglutinin that lead to changes in receptor-binding specificity have been described.[143] Changes at the single residue located in a pocket on the distal tip of the molecule allow for the haemagglutin to recognize different sugar linkages in glycoproteins.

Southern-bean Mosaic Virus. – Rossmann and co-workers[144] have described in detail the three quasi-equivalent subunits of Southern-bean mosaic virus and shown that they share the same conformation except for the five residues in the carboxy-terminal vestigial hinge. When examined in pairs most interactions were seen between the five-fold related subunits, although the quasi-three-fold and quasi-six-fold related subunits have nearly the same number of contacts. Interactions across the two-fold axis are far fewer. Structural possibilities for the assembly of the virus are discussed in detail, and in an appendix[145] a possible manner for the binding of viral RNA to the coat protein is discussed. A pattern of basic residues on the coat-protein inner surface is able to dock very accurately with a nine-base-pair double-helical A-RNA structure. A second appendix[146] has considered the accuracy of the electron-density map.

Alfalfa Mosaic Virus. – A $T = 1$ empty aggregate of alfalfa-mosaic-virus coat protein has been crystallized and the orientation of the subunits determined with the rotation function.[147] With a single heavy-atom derivative an electron-density map has been calculated at 0.45 nm resolution. The protein-coat shell lies between 5.8 nm and 9.5 nm radius and the subunit boundary can be easily traced. Each subunit has a central cavity reminiscent of the β-barrel seen in other spherical plant viruses, but the topology has not yet been determined unambiguously. The spherical particles have large holes at the five-fold axes, and there are substantial interactions across the two-fold and three-fold axes. In an

[142] A. J. Caton, F. L. Raymond, G. G. Brownlee, J. W. Yewdell, and W. Gerhard, *Biochem. Soc. Trans.*, 1983, **11**, 435.
[143] G. N. Rogers, J. C. Paulson, R. S. Daniels, J. J. Skehel, I. A. Wilson, and D. C. Wiley, *Nature (London)*, 1983, **304**, 76.
[144] M. G. Rossmann, C. Abad-Zapatero, M. A. Hermodson, and J. W. Erickson, *J. Mol. Biol.*, 1983, **166**, 37.
[145] M. G. Rossmann, R. Chandrasekaran, C. Abad-Zapatero, J. W. Erickson, and S. Arnott, *J. Mol. Biol.*, 1983, **166**, 73.
[146] A. Silva, M. G. Rossmann, and C. Abad-Zapatero, *J. Mol. Biol.*, 1983, **166**, 80.
[147] L. Fukuyama, S. S. Abdel-Meguid, J. E. Johnson, and M. G. Rossman, *J. Mol. Biol.*, 1983, **167**, 873.

appendix[148] a novel way of finding the sites of heavy-atom substitution in the alfalfa-mosaic-virus crystals is described.

Tomato Bushy Stunt Virus. – The structure of tomato bushy stunt virus has been described at 0.29 nm resolution.[149] The viral shell is composed of 180 protein subunits with three similar but distinct binding modes. The ability to adopt alternative conformations is due to localized interdomain hinges and multiple conformations available to surface residues. The structure of the particle has also allowed an explanation of the mechanism by which the subunits correctly position themselves. On 60 of the 180 polypeptide chains, 35 amino acid residues fold in an ordered way to form an internal framework for the structure. The RNA is packed tightly in the interior of the particle and interacts mainly with flexible parts of the subunits attached to the well ordered framework. This flexibility imposes the minimum restrictions on the folding of the RNA.

The location of divalent-cation-binding sites in the tomato bushy stunt virus has also been reported.[150]

Small Spherical Plant Viruses. – Rossmann and co-workers have quantitatively compared the structures of tomato bushy stunt virus (TBSV), southern-bean mosaic virus (SBMV), and satellite tobacco necrosis virus (STNV).[151] The organizations of the shell domains of TBSV and SBMV are identical. The wedge-shaped end of the subunit is closer to the five-fold or quasi-six-fold axes in all three viruses, but the packing about the two-folds and three-folds is quite different in STNV when compared with TBSV or SBMV. Conservation of threonines and serines at the wedge-shaped end of the subunit and of lysines and arginines at the protein–RNA interface is seen. Also seen conserved are hydrophobic residues inside the β-barrel, ionic interactions at the five-fold contacts, and an aspartate residue near the site that binds calcium in TBSV. These authors suggest a divergent evolutionary tree for these viruses.

Polyoma Virus. – Rayment and co-workers[152] have described the techniques and application of molecular replacement used in the determination of the structure of the polyoma-virus capsid at 2.25 nm resolution. Extensive refinement has shown that the 72 capsomeres are pentamers and are arranged with icosahedral symmetry.

12 Muscle and Muscle Proteins

Maéda has investigated the arrangement of myosin heads in relaxed crab muscle.[153] The results suggest a model in which the myosin heads are arranged in a helical

[148] M. A. Hermodson, *J. Mol. Biol.*, 1983, **167**, 891.
[149] A. J. Olson, G. Bricogne, and S. C. Harrison, *J. Mol. Biol.*, 1983, **171**, 61.
[150] J. Hogle, T. Kirchhausen, and S. C. Harrison, *J. Mol. Biol.*, 1983, **171**, 95.
[151] M. G. Rossmann, C. Abad-Zapatero, M. R. N. Murthy, L. Liljas, T. A. Jones, and B. Strandberg, *J. Mol. Biol.*, 1983, **165**, 711.
[152] I. Rayment, T. S. Baker, and D. L. D. Caspar, *Acta Crystallogr., Sect. B*, 1983, **39**, 505.
[153] Y. Maéda, *Nature (London)*, 1983, **302**, 69.

manner with four-fold symmetry around the thick-filament axis. Each head appears to be elongated, 16–18 nm long and 3–4 nm thick, and in resting muscle is centred 13.5 nm from the axis of the nearest thin filament. This suggests that the tip of the myosin head is very close to the surface of the thin filaments.

Poulsen and Lowy[154] have examined the small-angle *X*-ray scattering from myosin heads in frog skeletal muscles in both the relaxed and rigor states. In full-overlap rigor the disk is compressed in the diagonal direction, indicating that the myosin heads have a bent shape and a preferred orientation consistent with a 45° angle of attachment to actin.

Crystalline sheets of actin have been used to yield a three-dimensional reconstruction of the actin molecule.[155] This reconstruction shows actin to be an asymmetric, wedge-shaped molecule.

Freundlich and Squire[156] have examined the three-dimensional structure of insect flight-muscle M-band, and Huxley and co-workers[157] have used synchrotron radiation to examine changes in the *X*-ray-diffraction pattern from contracting muscle. The high intensity of the *X*-rays has allowed a time resolution of 1 ms or less and major changes in the 14.3 nm meridional reflection to be observed.

13 Membranes and Membrane Proteins

Purple Membrane. – Leifer and Henderson[158] have determined the structure of the orthorhombic form of the purple membrane at 0.65 nm resolution. The structure of each bacteriorhodopsin molecule consists of the seven rod-shaped features as seen in the native trigonal form. Additionally, the positions of some stretches of polypeptide chain linking the ends of adjacent rods have been seen.

Cytochrome Reductase. – The examination of membrane crystals of ubiquinol-cytochrome *c* reductase from *Neurospora* mitochrondria has been reported.[159] The structure of the cytochrome bc_1 complex has been determined at low resolution and compared with the structure of the whole cytochrome reductase. This has allowed tentative positions to be assigned to the subunits missing from the cytochrome bc_1 complex. Improved methods for the production of crystals of both ubiquinol–cytochrome *c* reductase and the cytochrome bc_1 subcomplex have been described.[160]

Neutron-scattering studies on mitochondrial ubiquinol–cytochrome *c* reductase and some of its subcomplexes have been published.[161] The length of the enzyme across the bilayer or the detergent micelle is between 15.0 and 17.5 nm and of the cytochrome bc_1 subcomplex is between 9.0 and 11.5 nm.

[154] F. R. Poulsen and J. Lowy, *Nature (London)*, 1983, **303**, 146.
[155] P. R. Smith, W. E. Fowler, T. D. Pollard, and U. Aebi, *J. Mol. Biol.*, 1983, **167**, 641.
[156] A. Freundlich and J. M. Squire, *J. Mol. Biol.*, 1983, **169**, 439.
[157] H. E. Huxley, R. M. Simmons, A. R. Faruqi, M. Kress, J. Bordas, and M. H. J. Koch, *J. Mol. Biol.*, 1983, **169**, 469.
[158] D. Leifer and R. Henderson, *J. Mol. Biol.*, 1983, **163**, 451.
[159] B. Karlsson, S. Hovmöller, H. Weiss, and K. Leonard, *J. Mol. Biol.*, 1983, **165**, 287.
[160] S. Hovmöller, M. Slaughter, J. Berriman, B. Karlsson, H. Weiss, and K. Leonard, *J. Mol. Biol.*, 1983, **165**, 401.
[161] S. J. Perkins and H. Weiss, *J. Mol. Biol.*, 1983, **168**, 847.

Porin. – The production of two-dimensional crystalline porin sheets has been reported,[162] and the three crystalline forms have been characterized. The different crystal forms can be correlated to different protein-to-phospholipid ratios.

Membranes. – Brisson and Wade[163] have described the 3.5 nm resolution structure of urothelial plasma membrane determined from negatively stained two-dimensional assays of membrane proteins. The results suggest that protein extensions form protrusions 5.0 nm from the extracellular side of the membrane. No structure is observed on the cytoplasmic side of the membrane, suggesting that porin is not a transmembrane protein.

X-Ray-diffraction studies on mitochondrial, microsomal, and pellicular membranes from *Tetrahymena* cells have been reported.[164] The cells have been grown at various temperatures, and the phase-transition temperatures of the membranes have been studied.

The use of *X*-ray- and neutron-diffraction studies to examine reconstituted sarcoplasmic reticulum membranes has been described.[165] The authors compare the separate calcium-pump protein profile with that of isolated sarcoplasmic reticulum membranes, and they have determined the time-average perturbation of the lipid bilayer by the incorporation of the calcium-pump protein.

14 Other Biological Structures

Collagen. – The use of synchrotron radiation to study the mechano-chemically induced molecular transformations of collagen fibres has been described,[166] and Fraser and co-workers[167] have collected new *X*-ray data from tendon collagen stained with phosphotungstic acid. For the first time a complete description of the unit cell has been possible. The authors show that none of the existing models for fibre structure accounts for all their results, and they have developed and tested alternative models.

Ribosomes and Ribosomal Components. – The 0.5 nm structural analysis of protein S5 from *Bacillus stearothermophilus* has been reported.[168] The molecule is essentially compact and only slightly elongated, with an axial ratio of about 1.5 :1. This varies quite considerably from the values previously obtained in solution.

The low-resolution structure of protein L30 from *B. stearothermophilus* ribosomes has also been described.[169] The molecule is compact, extending to

[162] D. L. Dorset, A. Engel, M. Häner, A. Massalski, and J. P. Rosenbusch, *J. Mol. Biol.*, **1983**, **165**, 701.
[163] A. Brisson and R. H. Wade, *J. Mol. Biol.*, 1983, **166**, 21.
[164] H. Nakayama, M. Goto, K. Ohki, T. Mitsui, and Y. Nozawa, *Biochim. Biophys. Acta*, **1983**, **730**, 17.
[165] L. Herbette, A. Scarpa, J. K. Blasie, C. T. Wang, L. Hymel, J. Seelig, and S. Fleischer, *Biochim. Biophys. Acta*, 1983, **730**, 369.
[166] Th. Nemetschek, K. Jelinek, E. Knörzer, E. Mosler, H. Nemetschek-Gansler, H. Riedl, and V. Schilling, *J. Mol. Biol.*, 1983, **167**, 461.
[167] R. D. B. Fraser, T. P. MacRae, A. Miller, and E. Suzuki, *J. Mol. Biol.*, 1983, **167**, 497.
[168] S. W. White, K. Appelt, J. Dijk, and K. S. Wilson, *FEBS Lett.*, 1983, **163**, 73.
[169] K. Appelt, J. Dijk, S. White, K. Wilson, and K. Bartels, *FEBS Lett.*, 1983, **160**, 72.

about 2.5–3.0 nm in each dimension, and two electron-dense features have been tentatively assigned as α-helices.

Three-dimensional reconstruction and averaging of 30S ribosomal subunits[170] and 50S ribosomal subunits[171] have been described. For the 30S particle a stained channel-like structure, possibly segments of the 16S RNA, has been found inside the particle.[170] A similar stained channel substructure has been found inside the 50S subunit.[171]

Kearney and Moore[172] have compared 30S and 50S subunits in their active and inactive conformational states. Using small-angle *X*-ray scattering they have shown that the differences between the active and inactive states are small, so small as to be beyond the reach of physical methods currently being used to examine these particles.

Proteins L1, L3, L4, and L23 from *E. coli* have had their shapes determined *in situ* by neutron small-angle scattering.[173] Using a novel technique for equalizing the neutron-scattering lengths of RNA and proteins it has been possible to produce 50S subunits containing one or two protonated proteins in a contrast matched, partially deuteriated ribosomal matrix. A good signal-to-noise ratio is obtained, and the radii of gyration and axial ratios for the four proteins have been determined. Distance measurements between several protein couples have also been performed.

X-Ray and neutron-scattering studies of 16S RNA have been described,[174] both complexed with ribosomal proteins and in isolated form. The shape of the 16S RNA molecule in the 30S complex is only slightly more compact than isolated 16S RNA. Together with other observations, the results suggest that the specific folding of 16S RNA is governed by intramolecular interactions, but the stabilization to the final compact structure requires some ribosomal proteins.

15 Small-angle Scattering

Viruses. – Neutron scattering has been used to determine a model for the distribution of protein and RNA in alfalfa mosaic virus.[175] The protein is located from 6.5 nm to 10.0 nm radius with the RNA uniformly distributed up to 6.5 nm radius. Only a very small part of the protein extends into the RNA, and *X*-ray scattering has been unable to find any short- or long-range order in the RNA.

X-Ray fibre diffraction of the nucleoprotein complex of Pf_1 phage in various hydration states has shown[176] that the helix pitch changes from 4.4 nm in the dry state to 5.5 nm in wet fibres. The unit rise between subunits in the helix does not appear to alter.

[170] V. Knauer, R. Hegerl, and W. Hoppe, *J. Mol. Biol.*, 1983, **163**, 409.
[171] H. Oettl, R. Hegerl, and W. Hoppe, *J. Mol. Biol.*, 1983, **163**, 431.
[172] K. R. Kearney and P. B. Moore, *J. Mol. Biol.*, 1983, **170**, 381.
[173] K. H. Nierhaus, R. Lietzke, R. P. May, V. Nowotny, H. Schulze, K. Simpson, P. Wurmbach, and H. B. Stuhrmann, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 2889.
[174] I. N. Serdyuk, S. Ch. Agalarov, S. E. Sedelnikova, A. S. Spirin, and R. P. May, *J. Mol. Biol.*, 1983, **169**, 409.
[175] G. T. Oostergetel, J. E. Mellema, and S. Cusack, *J. Mol. Biol.*, 1983, **171**, 157.
[176] G. G. Kneale and D. A. Marvin, *J. Mol. Biol.*, 1983, **171**, 229.

The kinetics of the self-assembly of brome mosaic virus capsid have been studied by *X*-ray and neutron diffraction.[177] The process appears to be very fast and to proceed through intermediate states.

Adenovirus type 2 has been studied by *X*-ray and neutron diffraction,[178] and it has been shown that the distance between the major coat proteins in the capsid is 10.0 nm. Neutron studies have allowed the radial distribution of DNA and protein to be described.

The use of electron micrographs and digital image processing has shown that the 'hexamer' tubes of polyma virus consist of paired pentameric capsomers.[179]

Tubulin. – The use of synchrotron radiation has allowed the assembly and disassembly of tubulin to be examined in a time-resolution study.[180] Several structurally distinct states were resolved during one assembly/disassembly cycle. The same authors[181] have studied *X*-ray patterns from solutions of tubulin rings and microtubules. The data are consistent with models in which the rings consist of coiled protofilaments, in agreement with some models but excluding others.

A three-dimensional reconstruction of tubulin sheets has suggested that the three-start helix of microtubules is right-handed rather than left-handed.[182]

***Sulfolobus* Cell-wall Protein.** – Crystallographic analysis of electron micrographs of pieces of the S-layer that surrounds the bacterium *Sulfolobus acidocaldarius* has been reported.[183] The asymmetric unit consists of several domains connected by strong hinges, and the authors suggest that the single species of polypeptide chain is in a highly extended conformation with much overlapping between different molecules. In an appendix[184] a least-squares method for determining structure factors in three-dimensional tilted-view reconstructions is described.

DNA–Protein Complexes. – Complexes of DNA with arginine-containing peptides have been studied by *X*-ray diffraction,[185] and it is concluded that the arginine interacts with DNA in its major groove. The authors suggest that protamine is bound to the major groove of DNA.

A structural analysis of T4 DNA helix destabilizing protein has been reported,[186] and the low-resolution model shows that the protein is 9.0 nm long and 2.0 nm in diameter.

Other Small-angle Studies. – *X*-Ray-absorption near-edge structure studies on the calcium-binding sites of troponin C have shown that all four sites have the calcium ion co-ordinated to carboxyl and carbonyl groups in a characteristic

[177] M. Cuillel, C. Berthet-Colominas, B. Krop, A. Tardieu, P. Vachette, and B. Jacrot, *J. Mol. Biol.*, 1983, **164**, 645.
[178] C. Devaux, P. A. Timmins, and C. Berthet-Colominas, *J. Mol. Biol.*, 1983, **167**, 119.
[179] T. S. Baker, D. L. D. Caspar, and W. T. Murakami, *Nature (London)*, 1983, **303**, 446.
[180] J. Bordas, E.-M. Mandelkow, and E. Mandelkow, *J. Mol. Biol.*, 1983, **164**, 89.
[181] E. Mandelkow, E.-M. Mandelkow, and J. Bordas, *J. Mol. Biol.*, 1983, **167**, 179.
[182] R. Schultheiss and E. Mandelkow, *J. Mol. Biol.*, 1983, **170**, 471.
[183] J. F. Deatherage, K. A. Taylor, and L. A. Amos, *J. Mol. Biol.*, 1983, **167**, 823.
[184] D. A. Agard, *J. Mol. Biol.*, 1983, **167**, 849.
[185] I. Fita, J. L. Campos, L. C. Puigjaner, and J. A. Subirama, *J. Mol. Biol.*, 1983, **167**, 157.
[186] H. Cohen and W. Chiu, *J. Mol. Biol.*, 1983, **169**, 235.

manner.[187] This geometry is distorted when magnesium ions are bound. Similar studies on parvalbumin are included.

Small-angle neutron-scattering studies of α_1-acid glycoprotein have shown[188] that it has a molecular weight of 37 000 and a radius of gyration that shows the molecule can exist in expanded and contracted forms.

Small-angle *X*-ray studies on adenosine triphosphatase from a thermophilic bacterium have shown[189] that the radius of gyration decreases on the binding of ADP. Similar studies on lobster arginine kinase[190] show similar contractions when nucleotides bind, suggesting a hinge motion of domains as seen for other phosphokinases.

Small-angle studies on cytosolic aspartate aminotransferase give no evidence[191] of major conformational change on the binding of pyridoxal 5′-phosphate to the apoenzyme, and cytochrome P450 has been shown[192] to have the same radius of gyration in the presence and absence of its substrate, camphor.

The solution structure of 7S globulin from *Phaseolus vulgaris* has been compared with the crystal structure of canavalin.[193] The considerable differences may be caused by the tryptic treatment of canavalin before crystallization.

The 11S globulins from sunflower and rape seeds have been studied[194] by small-angle *X*-ray scattering, and both molecules consist of six subunits arranged as a trigonal antiprism.

Small-angle studies on tryptophan synthase and its two subunits, α and β, have been reported,[195] and it seems that conformational changes of the subunits accompany the formation of the active complex.

16 Amino Acids and Peptides

Amino Acids and Small Peptides. – The structure of a dipeptide containing a thiopeptide linkage has been reported.[196] Except for differences between the carbonyl and the thioacyl bond lengths there are no significant differences between the oxopeptide and the thiopeptide unit.

Benedetti and co-workers[197] have examined the preferred conformation of the benzyloxycarbonyl amino group in peptides, and Suresh and Vijayan[198]

[187] A. Bianconi, A. Giovannelli, L. Castellani, S. Alema, P. Fasella, B. Oesch, and S. Mobilio, *J. Mol. Biol.*, 1983, **165**, 125.
[188] Z. Q. Li, S. J. Perkins, and M. H. Loucheux-Lefebre, *Eur. J. Biochem.*, 1983, **130**, 275.
[189] T. Furono, A. Ikegami, H. Kihara, M. Yoshida, and Y. Kagawa, *J. Mol. Biol.*, 1983, **170**, 137.
[190] C. Dumas and J. Janin, *FEBS Lett.*, 1983, **153**, 128.
[191] D. Vergé, A. Tardieu, and M. Arrio-Dupont, *FEBS Lett.*, 1983, **154**, 277.
[192] B. A. Lewis and S. G. Sligar, *J. Biol. Chem.*, 1983, **258**, 3599.
[193] P. Plietz, G. Damaschun, J. J. Müller, and B. Schlesier, *FEBS Lett.*, 1983, **162**, 43.
[194] P. Plietz, G. Damaschun, J. J. Müller, and K.-D. Schwenke, *Eur. J. Biochem.*, 1983, **130**, 315.
[195] P. Wilhelm, I. Pilz, A. N. Lane, and K. Kirschner, *Eur. J. Biochem.*, 1983, **129**, 51.
[196] T. F. M. La Cour, H. A. S. Hansen, K. Clausen, and S.-O. Lawesson, *Int. J. Pept. Protein Res.*, 1983, **22**, 509.
[197] E. Benedetti, C. Pedore, C. Toniolo, M. Dudek, G. Némethy, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1983, **21**, 163.
[198] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **21**, 223.

have reported a head-to-tail structure for L-arginine acetate. The structure of L-ornithine-L-aspartate hemihydrate reveals the unlike molecules forming separate two-fold helices and shows head-to-tail alignment of two types of amino acid.[199] Head-to-tail sequences have also been seen[200] in the crystal structure of L-lysine acetate, and a novel structure of the lysine side chain in the presence of sulphate ion has been described.[201] The structure of L-aspartate with a neutral α-carboxyl group and a deprotonated side-chain carboxyl group has been determined.[202]

Eggleston and Hodgson have reported the structures of the acidic dipeptides L-alanyl-L-aspartate,[203] α-L-glutamyl-L-aspartate,[203] and α-L-leucyl-L-glutamate;[204] the structure of L-arginyl-L-glutamate has also been determined.[205]

The crystal structure of α-methyl-*m*-tyrosine, which acts as a substrate for the enzymes of norepinephrine synthesis leading to the analogue metraminol, has been reported by Satyshur and Rao.[206]

Larger Peptides. – Crystals of polyglycine, molecular weight 5000, have been examined, and the molecule is shown to have a folded β-structure.[207] A decapeptide containing five α-aminoisobutyric acid residues has been crystallized and shown to adopt a right-handed 3_{10} helical conformation;[208] the structure of a cyclic octapeptide, cyclo(L-Pro-Sar)$_4$, has been reported.[209]

The structure of [Leu^5]enkephalin has been determined[210] and the structure described in detail.[211] The asymmetric unit contains four independent molecules of enkephalin and much water and dimethyl formamide solvent. All four enkephalin molecules have extended peptide backbones with the tyrosine, phenylalanine, and leucine side chains above and below the plane of the backbone. The four molecules form a slightly irregular antiparallel β-sheet, and it is suggested that, unlike previous models of a hairpin loop structure, the extended conformation may be the form that binds to the opiate μ-receptors.

The structure of a tripeptide part of enkephalin, t-butoxycarbonyl-glycyl-glycyl-L-phenylalanine ethyl ester, has been described[212] and shows that the molecule adopts a β-bend conformation around the glycylglycyl bond.

[199] D. M. Salunke and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 154.
[200] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 617.
[201] S. Capasso, C. A. Mattia, L. Mazzarella, and A. Zagari, *Acta Crystallogr., Sect. C*, 1983, **39**, 281.
[202] C. G. Suresh and M. Vijayan, *Int. J. Pept. Protein Res.*, 1983, **22**, 176.
[203] D. S. Eggleston and D. J. Hodgson, *Int. J. Pept. Protein Res.*, 1983, **21**, 288.
[204] D. S. Eggleston and D. J. Hodgson, *Acta Crystallogr., Sect. C*, 1983, **39**, 75.
[205] J. Pandit, T. P. Seshadri, and M. A. Viswamitra, *Acta Crystallogr., Sect. C*, 1983, **39**, 1669.
[206] K. A. Satyshur and S. T. Rao, *Acta Crystallogr., Sect. C*, 1983, **39**, 1672.
[207] S. Muñoz-Guerra, J. Puiggalí, A. Rodríguez, and J. A. Subirana, *J. Mol. Biol.*, 1983, **167**, 223.
[208] A. K. Francis, M. Iqbal, P. Balaram, and M. Vijayan, *FEBS Lett.*, 1983, **155**, 230.
[209] T. Shimizu, K. Ueno, Y. Tanaka, and K. Tsuda, *Int. J. Pept. Protein Res.*, 1983, **22**, 231.
[210] I. L. Karle, J. Karle, D. Mastropaolo, A. Camerman, and N. Camerman, *Acta Crystallogr., Sect. B*, 1983, **39**, 625.
[211] A. Camerman, D. Mastropaolo, I. Karle, J. Karle, and N. Camerman, *Nature (London)*, 1983, **306**, 447.
[212] T. Ishida, N. Tanabe, and M. Inoue, *Acta Crystallogr., Sect. C*, 1983, **39**, 110.

The crystal structure of a protected analogue of the C-terminal dipeptide of gramicidin A has been reported.[213] One of the molecules gives the first evidence of a β-turn conformation fused with the oxy analogue of an α-turn. N.m.r. studies[214] suggest that the β-turn conformation may well be the favoured conformation in dilute solution.

Cotrait[215] has described the structure of Pro-Arg-Arg-Pro, a neurotensin fragment, and of Arg-Pro-Lys-Pro, a substance P fragment, and Admiraal and Vos[216] have reported the structure of Met-Glu-His-Phe, a tetrapeptide fragment of adrenocorticotropic hormone.

Benedetti and co-workers[217] have reported the structure of the protected 1–3 segment of the antibiotics alamethicin and hypelcin.

Islam and Neidle have described[218] the use of different potential-energy functions for investigating the structure of daunomycin. The calculated structures are close to those seen in the crystalline state, suggesting that these methods may well be applicable to amino acid and peptide structures.

17 Protein Conformations and Structure Prediction

Protein Conformations. – Benedetti and co-workers[219] have analysed the side-chain conformations of 321 residues in 258 crystal structures and have summarized the preferred side-chain torsional angles. These torsional-angle distributions have been compared with those seen in proteins and have been shown to be similar. The characteristics of side-chain contact-frequency distributions have been examined,[220] and it shows that, apart from lysine and arginine, the mean number of contacts is proportional to the side-chain surface area. Amino acids in β-sheets show a greater number of contacts than those in α-helical regions.

Viswanadham and Sundaram[221] have examined the specificity of clustering of amino acids in cytochrome *c* and myoglobin and have shown that the specificity of the cluster increases for the higher taxonomic orders. Kolaskar and Ramabrahmam[222] have examined the conformational properties of pairs of amino acids, and Suresh and Vijayan[223] have described the occurrence and geometrical features of head-to-tail associations in amino acid crystal structures. The computed conformational states of the 20 naturally occurring amino acids have been reported.[224]

[213] E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo, and G. M. Bonova, *Biochem. Biophys. Res. Commun.*, 1983, **112**, 1056.
[214] G. M. Bonova, C. Toniolo, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone, and C. Pedone, *J. Biol. Chem.*, 1983, **258**, 14 725.
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[222] A. S. Kolaskar and V. Ramabrahmam, *Int. J. Pept. Protein Res.*, 1983, **22**, 83.
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[224] M. Vásquez, G. Némethy, and H. A. Scheraga, *Macromolecules*, 1983, **16**, 1043.

Newton[225] has considered theoretical aspects of the OH···O hydrogen bond and the role it plays in structural and kinetic phenomena, and Taylor and Kennard[226] have compared *X*-ray- and neutron-diffraction results for the N–H···O=C hydrogen bond. Barlow and Thornton[227] have examined ion pairs in proteins and shown that about one third of charged residues are involved in ion pairs and 76% of these stabilize the tertiary structure. Only 17% of ion pairs are buried, and conservation of the interactions is generally low.

Levitt has described the techniques of computer simulation of the atomic motion of bovine pancreatic trypsin inhibitor[228] for up to 132 picoseconds and has analysed their computed motions.[229]

Thornton and Sibanda[230] have analysed the structural, dynamic, and functional aspects of amino- and carboxy-terminal regions in known protein structures. They show that the terminal regions are usually located on the surface of the protein, are accessible to the solvent, and are often flexible. Terminal regions in single-domain proteins and within individual domains of larger proteins tend to be in close proximity.

Sundaram and co-workers[231] have shown how the sequence of local conformations at α-carbon atoms can be considered as an informational message string and used to analyse repeating, intricate structural features. Viswanadhan and Sundaram[232] have considered structural conservation in globular proteins, and Sweet and Eisenberg[233] have shown how the similarity of protein structures can be examined by comparing the patterns of hydrophobicity in the sequences.

Braun[234] has shown how short- and long-range handedness in protein structures can be represented by signed distance maps, and Yuschok and Rose[235] have analysed the hierarchic organization of a set of 1000 simulated chain folds, concluding that results previously reported for actual protein structures were significant.

Rose and co-workers[236] have found that reverse turns can occur buried in the interior of proteins but, when they do, one or more solvent molecules are found in a hydrogen-bonded complex with main-chain atoms of the turn residues. These water molecules appear to function as an integral part of the protein structure. Flinta and co-workers[237] have shown that the distribution of polar residues in randomly generated helices suggests that the bias seen in real helices is far from statistically significant.

[225] M. D. Newton, *Acta Crystallogr., Sect. B*, 1983, **39**, 104.
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[228] M. Levitt, *J. Mol. Biol.*, 1983, **168**, 595.
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Chothia has considered the coiling of β-sheets and the conditions necessary to form strongly twisted sheets.[238] Torsional angles that should lead to twisted sheets are seen to be fulfilled by four proteins containing such sheets. The role of interchain interactions in stabilizing the right-handed twist of β-sheets has been examined.[239] All computed minimum-energy β-sheets were found to have right-handed twists of a size corresponding to the mean twist of observed β-sheets.

Go and Abe[240] have discussed the randomness of the protein-folding process, and Go and Kano[241] have described a technique for using a markoffian process to explain protein folding.

Structure Predictions. – Kabsch and Sander[242] have tested the three most widely used methods for protein-structure prediction on 62 proteins of known structure. None of the methods predicts better than 56% of the residues correctly for a three-state model. Rawlings and co-workers[243] have described a computerized secondary-structure prediction program based on the algorithm of Chou and Fasman.

Cohen and co-workers[244] describe an algorithm for assigning secondary structure in α/β proteins that identifies turns with a 98% accuracy. Although the assignments for a given sequence are not unique, at least one of the structural assignments bears a close resemblance to the true structure. Taylor and Thornton have described[245] a prediction technique based on the recognition of super-secondary structures, initially using the β–α–β unit. The method correctly identified 70% of the β–α–β units in 16 α/β proteins and led to a 7.5% average improvement over the original secondary-structure predictions.

Levitt[246] has reported the modelling of protein folding by restrained energy minimization and molecular dynamics, and a prediction of the secondary structure of chloroplast thylakoid membrane protein[247] suggests that the molecule consists of seven transmembrane helices connected by exposed turns. This model is the same as that seen in bacteriorhodopsin and bovine rhodopsin, suggesting that the seven-helical bundle may prove to be a conserved feature of integral membrane proteins.

Argos and Siezen[248] have described a method for detecting structural homology from amino acid sequences, and Dijkstra and co-workers[249] have shown that polypeptides with very similar sequences can show distinctly different con-

[238] C. Chothia, *J. Mol. Biol.*, 1983, **163**, 107.
[239] K.-C. Chou, G. Némethy, and H. A. Scheraga, *J. Mol. Biol.*, 1983, **168**, 389.
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formations. The observation suggests that structure predictions based on short-segment similarities alone might give incorrect results.

Hollecker and Creighton[250] have described the folding pathways of two protease-inhibitor homologues from black-mamba venom, and Argos and co-workers[251] have reported the possible identification of a nucleotide-binding domain in the folding of phosphoribosyltransferases. Possible folding patterns for urokinase and tissue-type plasminogen activator have been predicted[252] by alignment with known serine protease structures, and Pernollet and Mossé[253] have reported structure predictions of seed storage proteins. Predictions have also been reported for the structures of plant lectins,[254] p21 protein and its cancer-associated variant,[255] and neurotensin, xenopsin, and bradykinin binding to mast-cell receptors.[256]

PART III: Conformation and Interaction of Peptides and Proteins in Solution

Edited by R. H. Pain, with contributions by D. J. Barlow, J. O. Baum, S. H. Bell, T. Brittain, S. Craig, D. P. E. Dickson, M. L. J. Drummond, J. L. Finney, P. D. Jeffrey, A. Mathews, L. W. Nichol, J. C. Smith, R. M. Stephens, J. M. Thornton, and D. J. Winzor

1 Theoretical Aspects of Protein Conformation

Contributed by D. J. Barlow, J. O. Baum, M. L. J. Drummond, J. L. Finney, J. C. Smith, and J. M. Thornton

We report on publications available at the end of February 1984. This year's increased coverage of protein dynamics reflects significantly increased activity in this field.

Potential-energy Functions. – No new potentials have been published, but several have been extended or updated.[1-5] A simpler treatment of the nitrogen

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[254] K. W. Olsen, *Biochim. Biophys. Acta*, 1983, **743**, 212.
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[256] L. U. Podinsh, Ya. R. Betinsh, G. V. Nikifovovich, and G. I. Chipens, *FEBS Lett.*, 1983, **153**, 25.
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[4] M. Levitt, *J. Mol. Biol.*, 1983, **168**, 595.
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parameters of the ECEPP model gave an improved fit to oligopeptide crystal data,[1,2] while an O—H···O hydrogen-bond potential was added to the MM2 model[6] by fitting to quantum-mechanical water dimer energies.[3] Other hydrogen-bond representations include an r^{-n} even-power series fitted to simple hydrogen-bonded dimers,[7] and two angle-modified 12-6 functions were used in a molecular-dynamics simulation (see below) by Levitt.[4] He also developed a 'soft atom' van der Waals potential for energy minimization in which the repulsion never exceeds a given value.[5] Andrews *et al.*[8] fitted an intermolecular glycine potential to experimental vibrational frequencies. Bond dipoles and polarizabilities have been used to obtain charge and dipole models for use in conjunction with MM2.[9] Birge *et al.*[10] describe a polarization-catastrophe-avoiding dipole-polarizability model.

Energy Calculations and Conformational Analysis. – An energy-minimization technique based on distance geometry and combinatorial optimization has been developed by Havel *et al.* and used to find a putative global minimum and several local minima for *N*-methyl glycinamide.[11,12] Its heavy computational demands seem to preclude an easy extension to proteins. Saruyama[13] showed that for a general helical polymer the crystal structure will not necessarily be that with the minimum intramolecular energy. Fraga[14] added water molecules one by one to amino acids, each time energy-minimizing the system to investigate amino acid solvation.

The peptide fragments Pro-Arg-Arg-Pro of neurotoxin and Arg-Pro-Lys-Pro of substance P were found capable of many configurations of nearly equal energy.[15] Similar results for Boc-Aib-Pro-Pro-NHMe were used to interpret *X*-ray and n.m.r. data.[16] A conformational space search for Ac-Asn-Ala-Thr-NH_2 yielded some 800 local minima.[17] Some 21 of these, which would be present at 25 °C as at least 1% of a sample, have structural similarities that were postulated to be required by a substrate for *N*-glucosylation. Five low-energy conformers were postulated for neurotensin and they were used with proposed structures for xenopsin and bradykinin to try to understand structurally the observed competitive binding.[18] The difference in activity of an oncogene product and its normal homologue was investigated by predicting the structure

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[17] J. M. Ricart, J. J. Perez, M. Pons, and E. Giralt, *Int. J. Biol. Macromol.*, 1983, **5**, 279.
[18] L. U. Podinsh, Ya. R. Betinsh, G. V. Nikiforovich, and G. I. Chipens, *FEBS Lett.*, 1983, **153**, 25.

of a decapeptide containing the one amino acid by which they differ.[19] The energetics of Na^+ ions moving through the channel of gramicidin A were studied by Pullman and Etchebest.[20]

In a study of the interactions that stabilize twisted β-sheets, recently reviewed by Salemme,[21] Chou *et al.*[22,23] concluded that for small side chains intrachain energies dominate whereas for larger side chains interchain energies seem more important. The packing of two equivalent rigid α-helices was investigated, and ten low-energy arrangements were found within 6 kcal mol^{-1} of the global minimum.[23] Eight of these correspond to packings observed by Chothia.[24] Changes in bond lengths and angles in α and β conformations were examined by Scarsdale *et al.*[25]

Using crude energy calculations, Kolchanov *et al.*[26] identified single amino acid changes on the GH helices of myoglobin that would destabilize the tertiary structure, and they found agreement with sequence data. Energy-minimized structures of bovine pancreatic trypsin inhibitor (BPTI) and a chemical modification showed appreciable differences only near the sites of modification.[27] The theoretical structural changes agreed with n.m.r. results.

Levitt[5] energy-minimized BPTI from various random starting points, restraining the disulphide bridges and secondary-structure hydrogen bonding. Those resulting structures that deviated least from the *X*-ray structure were of lowest energy. Mao and McCammon[28] calculated the conformational-energy change associated with hinge bending in L-arabinose-binding protein. The energy difference was not great, and solvation changes on the cleft surfaces were important to the energetics of ligand binding. Wipff *et al.*[29] found that both protein and substrate had to be relaxed if stereoselectivity was to be observed in α-chymotrypsin.

Intermolecular Calculations. – The empirically calculated electrostatic potentials (ESP) of flavodoxin and cytochrome *c* may orient the approaching proteins to give a good reaction complex.[30] This is due to both molecules having highly asymmetric potentials. Similar calculations on Zn,Cu superoxide dismutase[31] suggest a similar role for the ESP.

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[31] E. D. Getzoff, J. A. Tainer, P. K. Weiner, P. A. Kollman, J. S. Richardson, and D. C. Richardson, *Nature (London)*, 1983, **306**, 287.

Active-site Calculations and Environmental Effects. – Although quantum mechanics can be applied to a *small* number of atoms in the active site, other methods are necessary to handle the perturbing effects of surrounding protein and solvent environments. Detailed reviews are given by Tapia[32] and by Simkin and Sheikhet.[33] The self-consistent reaction-field theory of Tapia and Goscinski,[34] previously applied to liver alcohol dehydrogenase,[35] models the solvent ESP, assuming a dipole field. In a study of the rhodopsin system Raudino *et al.*[36] extended this to a multipole expansion but required twenty terms for adequate convergence. The geometrically more realistic model of Thole and van Duijnen[37] self-consistently treats the environment as a set of interacting point polarizabilities. This method, previously used to explain the inactivity of thiol-subtilisin,[38] has now been applied to papain and actinidin.[39,40] The experimentally observed ion pair is argued to be stabilized by the α-helix dipole field, a claim challenged by Lavery *et al.*,[41] who, on the basis of their many-centre multipole expansion of the electrostatic field (fitted to minimal basis-set SCF results), conclude that the stabilizing effects of other static charges are of the same order of magnitude.

In other calculations, Angyan and Naray-Szabo[42] concluded that the protein ESP stabilizes charge separation in the reactive site of the serine proteases. That a buried aspartate was important to this stabilization[43] demonstrates the importance of including protein atoms *not* in the reactive site, as did the study by Krishtalik *et al.*[44] of the electric field of α-chymotrypsin. From energy calculations on the relevant *X*-ray structures, Warshel *et al.* concluded that the cytochrome *c* environment is much more favourable for electron transfer than is an aqueous environment.[45,46]

Analysis and Prediction of Secondary Structure. – The recent explosion in the number of protein sequences from cDNA work has led to the wide-spread

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application of existing methods for secondary-structure prediction.[47,51] Computer versions of the algorithms have been described,[72,73] and the accuracy of the methods has been assessed.[72] The relationship between sequence and secondary structure continues to be studied,[74–84] and novel algorithms, with improved predictive power, have been developed.

Application of Existing Methods for Prediction. Existing methods for prediction of secondary structure have been applied to a wide variety of proteins, including (*a*) globular proteins (cystatin,[47] seed storage proteins,[48] bovine β-lactoglobulin B,[49] α-fetoprotein,[50] the urotensin family of peptides,[51] adenovirus protein VII,[52] *E. coli* LamB,[53] repA[54] and mutant recA[55] proteins, mammalian metallothionein,[56] the tryptic —SH peptide of thermitase,[57] preprostomatostatin,[58] seminal plasmin,[59] yeast flavocytochrome *b*2,[60] *Bacillus stearothermophilus* lactate dehydrogenase,[61] and several signal sequences[62]), (*b*) fibrous proteins (usually using McLachlan's Fourier-transform method) (mouse-epidermal[63] and fowl-feather[64] keratin, hamster-lens vimentin,[65] nematode myosin,[66] and adenovirus fibre protein[67]), and (*c*) membrane proteins (cytochrome oxidase,[68] the

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[68] K. G. Welinder and L. Mikkelsen, *FEBS Lett.*, 1983, **157**, 233.

acetylcholine receptor subunits,[69] thylakoid membrane protein,[70] and phenobarbitol-induced cytochrome P450[71]).

Support for the predicted models has been sought in experimental data,[47,49,51,56,58,63,67] and ambiguities in predictions have been reduced by use of several homologous sequences for the polypeptide studied.[48,51,53,55–58,61,62,64,68] Although many predictions have been derived using only a single algorithm,[48,50,52–57,59,71] generally that due to Chou and Fasman,[88] other workers have produced consensus predictions using several algorithms.[47,49,51,58,60,64,67,70] Kabasch and Sander[72] have demonstrated the limitations of the existing prediction methods and have implemented computer versions of several existing algorithms.[72] A computer version of the Chou and Fasman method is also described by Rawlings *et al.*[73] Kabsch and Sander[74] have developed an automatic pattern-recognition program to identify secondary structural elements on the basis of hydrogen bonding, thus providing a much awaited dictionary of protein secondary structures.

Analysis of Secondary Structure. Through an analysis of the virtual bond and torsion angles for proteins, Soman and Ramakrishnan[75] have identified a number of rare helical conformations. Blundell *et al.*[76] have studied the distortion of α-helices caused by solvent and polar side-chain atoms and have related their findings to helix curvature. The amphipathic character of helices has been considered by Flinta *et al.*[77] and by Hamed and Mattice.[78] A new supersecondary structure, the αα-corner, has been identified by Effimov.[79]

Rose *et al.*[80] have shown that there are always buried water molecules associated with the main chain of the residues in interior turns. Salemme[21] has presented a comprehensive survey of the structural properties of β-sheets. Kolaskar and Ramabrahmam[81] have shown that the conformational preferences for pairs of amino acids are not a simple function of the preferences of the individual amino acids. Sundaram *et al.*[82] have, following Robson and Pain,[83]

[69] M. Noda, H. Takahashi, T. Tanabe, M. Toyosato, S. Kikyotani, Y. Furutani, T. Hirose, H. Takashima, S. Inayama, T. Miyata, and S. Numa, *Nature (London)*, 1983, **302**, 528.
[70] J. K. Rao, P. A. Hargrave, and P. Argos, *FEBS Lett.*, 1983, **156**, 165.
[71] G. E. Tarr, S. D. Black, V. S. Fujita, and M. J. Coon, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 6552.
[72] W. Kabsch and C. Sander, *FEBS Lett.*, 1983, **155**, 179.
[73] N. Rawlings, K. Ashman, and B. Wittman-Liebold, *Int. J. Pept. Protein Res.*, 1983, **22**, 515.
[74] W. Kabsch and C. Sander, *Biopolymers*, 1983, **22**, 2577.
[75] K. J. Soman and C. Ramakrishnan, *J. Mol. Biol.*, 1983, **170**, 1045.
[76] T. Blundell, D. Barlow, N. Borkakoti, and J. Thornton, *Nature (London)*, 1983, **306**, 281.
[77] G. Flinta, G. Von Heijne, and J. Johansson, *J. Mol. Biol.*, 1983, **168**, 193.
[78] M. M. Hamed and W. L. Mattice, *Biopolymers*, 1984, **23**, 201.
[79] A. V. Effimov, *FEBS Lett.*, 1984, **166**, 33.
[80] G. D. Rose, W. B. Young, and L. M. Gierasch, *Nature (London)*, 1983, **304**, 654.
[81] A. S. Kolaskar and V. Ramabrahmam, *Int. J. Pept. Protein Res.*, 1983, **22**, 83.
[82] K. Sundaram, V. N. Viswanadhan, and R. D. McElroy, *Int. J. Pept. Protein Res.*, 1983, **21**, 107.
[83] B. Robson and R. H. Pain, *Biochem. J.*, 1974, **141**, 853.

applied information theory to study the relationship between sequences and protein shape. Wuilmart and Urbain[84] have looked for periodicity in protein sequences and discuss their results in relation to the evolution of the molecules.

New Methods for Prediction. Taylor and Thornton[85] and Cohen *et al.*[86] both employ algorithms for pattern recognition by means of templates. Whereas Cohen *et al.* use a number of different templates to search for secondary structural elements, Taylor and Thornton use only one template, with variable length. Accurate prediction of the turn sites in a sequence is critical to the algorithm of Cohen *et al.*, and they claim a 98% success rate. Neither of the methods is designed solely to predict secondary structure; rather they locate sequences that may fold to form $\beta\alpha\beta$ super-secondary structure. A different new prediction algorithm due to Wako *et al.*[87] is based on a statistical model of protein conformation. As the statistical weights used in the algorithm are strongly dependent upon the proteins used in the database, the accuracy of their predictions for proteins not included in the database is quite poor.

Prediction of Tertiary Structure. – Where the three-dimensional structure for a related molecule has been determined crystallographically, the known structure may be used as a starting model for building the unknown structure. Using this approach, Wierenga and Hol[89] have derived a structure for the 37 N-terminal residues of human-bladder P21 protein, based on the $\beta\alpha\beta$ FAD-binding domain of *p*-hydroxybenzoate hydroxylase. Models have also been constructed by Blundell *et al.*[90] for mouse submaxillary renin (based on *endothia* pepsin), by Inana *et al.*[91] for murine β23 crystallin (based in γII crystallin), by Olsen[92] for various plant lectins (based on concanavalin A), and by Takio *et al.*[93] for cathepsins B and H (based on actinidin and papain).

The majority of the remaining predictions have involved prior prediction of secondary structure and have been made either for fibrous proteins,[63,67] where the coiled-coil structure is likely, or for membrane proteins,[68–70] where the main consideration is the arrangement of transmembrane helices. The fibrous and membrane proteins studied are the same as those subjected to secondary-structure prediction (see above), with the exception of cytochrome P450 and addition of acetylcholine receptor subunits.[94] The study of the histone core

[84] C. Wuilmart and J. Urbain, *Eur. J. Biochem.*, 1984, **139**, 35.
[85] W. R. Taylor and J. M. Thornton, *J. Mol. Biol.*, 1983, **173**, 487.
[86] F. E. Cohen, R. M. Abarbanel, I. D. Kuntz, and R. J. Fletterick, *Biochemistry*, 1983, **22**, 4894.
[87] H. Wako, N. Saito, and H. A. Scheraga, *J. Protein Chem.*, 1983, **2**, 221.
[88] P. Y. Chou and G. D. Fasman, *Biochemistry*, 1974, **13**, 222.
[89] R. K. Wierenga and W. G. J. Hol, *Nature (London)*, 1983, **302**, 842.
[90] T. Blundell, B. L. Sibanda, and L. Pearl, *Nature (London)*, 1983, **304**, 273.
[91] G. Inana, J. Piatigorsky, B. Norman, C. Slingsby, and T. Blundell, *Nature (London)*, 1983, **302**, 310.
[92] K. W. Olsen, *Biochem. Biophys. Acta*, 1983, **743**, 212.
[93] K. Takio, T. Towatari, N. Katunuma, D. C. Teller, and K. Titani, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 3666.
[94] E. M. Kosower, *FEBS Lett.*, 1983, **155**, 245.

protein[95] is strictly a quaternary prediction. A tertiary-structure prediction for the small protein metallothionein[56] was aided by the constraints imposed by its metal-ion-binding sites.

Analysis of Tertiary and Quarternary Structure. – The majority of these analyses can be broadly classified into (*a*) comparisons between related molecules,[96–102] (*b*) searches for structural patterns of possible predictive value,[26,103–111,116,117] and (*c*) new ways of representing the details of protein structure.[119,121–123] A smaller number of studies investigate the mechanism of protein folding[119,121,125] and the relationship between gene and protein structure.[91,92,97,112,115]

Comparison of Structures. The traditional approach of comparing molecules by least-squares methods has been used by several authors.[96–99] Hill *et al.*[96] have considered the structural homology between five microbial ribonucleases and have related their findings to the evolutionary relationships between the prokaryotic and eukaryotic enzymes. Grutter *et al.*[97] have given similar considerations to the goose, hen, and virus forms of lysosyme. Comparisons made between the porcine and bovine forms of phospholipase A2[98,99] and between the nucleotide-binding domains of *p*-hydroxybenzoate hydroxylase and glutathione reductase[100,101] have demonstrated that similar sequences may adopt quite different conformations.

The only new method proposed for comparing three-dimensional structures involves measuring the similarity in protein structures by correlating their sequence hydrophobicities.[102]

Analysis of Structures. With the improvements in resolution of protein structure, the consequent more accurate positioning of side-chain atoms has allowed the analysis of side-chain interactions to be tackled with more confidence. Most studies have concentrated on the distributions of specific groups within a protein's structure. A survey of ion pairs has been made by Barlow and Thornton,[103] considering their flexibility, conformation, accessibility, and conservation, and Rashin and Honig[104] have studied the environments of ionizable groups. Both groups show that charges are rarely buried in a protein and that where they are it is probably for functional rather than structural reasons. From

[95] C. C. Chipev, *Int. J. Biol. Macromol.*, 1983, **5**, 10.
[96] C. Hill, G. Dodson, U. Heinemann, W. Saenger, Y. Nakamura, M. Nakamura, K. Nakamura, S. Borisov, G. Tischenko, K. Polyakov, and S. Pavlovsky, *Trends Biochem. Sci.*, 1983, **8**, 364.
[97] M. G. Grutter, L. H. Weaver, and B. W. Matthews, *Nature (London)*, 1983, **303**, 828.
[98] B. W. Dijkstra, R. Renetseder, K. H. Kalk, W. G. J. Hol, and J. Drenth, *J. Mol. Biol.*, 1983, **168**, 163.
[99] B. W. Dijkstra, W. J. Weijer, and R. K. Wierenga, *FEBS Lett.*, 1983, **164**, 25.
[100] R. K. Wierenga and J. Drenth, *J. Mol. Biol.*, 1983, **167**, 725.
[101] W. J. Weijer, J. Holfsteenge, J. J. Bientema, R. K. Wierenga, and J. Drenth, *Eur. J. Biochem.*, 1983, **133**, 109.
[102] R. M. Sweet and D. Eisenberg, *J. Mol. Biol.*, 1983, **171**, 479.
[103] D. J. Barlow and J. M. Thornton, *J. Mol. Biol.*, 1983, **168**, 867.
[104] A. A. Rashin and B. Honig, *J. Mol. Biol.*, 1983, **173**, 515.

an analysis of the frequency distributions of side-chain contacts per residue, Bohacek *et al.*[105] have shown that the mean number of contacts is greater for β-sheet than for α-helical residues. They suggest also that cationic (as opposed to anionic) groups may play the major part in solvating the protein surface. The spatial placement of residues has been studied by Prabhakaran,[106] and Viswanadham and Sundaram[107,108] have looked at the clustering of residues in homologous proteins. Thornton and Sibanda[109] have investigated the distribution of N- and C-termini, considering proximity, accessibility, domain-linking, and function.

Olsen[110] has analysed the mesophilic and thermophilic forms of glyceraldehyde 3′-phosphate dehydrogenase and concludes that improved hydrophobic and electrostatic quaternary interactions stabilize the thermophilic tetramers. Gariepy and Hodges[111] relate the folding behaviour of EF hands to the mechanism of action of troponin C and calmodulin.

Few reports have analysed the relationship between gene and protein structure. Inana *et al.*[91] deal with the relation of intron–exon boundaries to their predicted structure for murine β23 crystallin, and Go[112] uses an algorithm for locating 'modules' (or compact structural units) in proteins to demonstrate the same relation for hen egg-white lysosyme. Go[112] also shows that the catalytic groups of the enzyme are located within the same module, whereas the substrate-binding sites are located in different modules. The correspondence between exons and protein domains has also been considered for other lysozymes,[97] dihydrofolate reductase,[113] the serine proteases,[113] mouse immunoglobulins,[114] and several lectins.[92] Craik *et al.*[113] discuss how sliding of intron–exon junctions along the genetic sequence may account for variation in the length of peptide sequences within protein families. A full discussion of evolutionary processes in relation to the tertiary structure of proteins is presented by Bajaj and Blundell.[115]

Chothia *et al.*[116] have considered the conformational changes that accompany shifts in the relative orientations of packed helices.

Using the observed frequencies of amino acid side-chain contacts, Roberts and Bohacek[117] have developed an automatic procedure to obtain the probability for a given side-chain group on a receptor interacting with a complementary group on a hormone.

[105] R. S. Bohacek, B. Davison, and D. C. Roberts, *Int. J. Pept. Protein Res.*, 1983, **21**, 21.
[106] M. Prabhakaran, *J. Theor. Biol.*, 1984, **106**, 25.
[107] V. N. Viswanadhan and K. Sundaram, *Int. J. Pept. Protein Res.*, 1983, **22**, 73.
[108] V. N. Viswanadhan and K. Sundaram, *Int. J. Pept. Protein Res.*, 1983, **21**, 190.
[109] J. M. Thornton and B. L. Sibanda, *J. Mol. Biol.*, 1983, **167**, 443.
[110] K. W. Olsen, *Int. J. Pept. Protein Res.*, 1983, **22**, 469.
[111] J. Gariepy and R. S. Hodges, *FEBS Lett.*, 1983, **160**, 1.
[112] M. Go, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 1964.
[113] C. S. Craik, W. J. Rutter, and R. Fletterick, *Science*, 1983, **220**, 1125.
[114] S. Tonegawa, *Nature (London)*, 1983, **302**, 575.
[115] M. Bajaj and T. Blundell, *Annu. Rev. Biophys. Bioeng.*, 1983, **13**, 453.
[116] C. Chothia, A. M. Lesk, G. G. Dodson, and D. C. Hodgkin, *Nature (London)*, 1983, **302**, 500.
[117] D. C. Roberts and R. S. Bohacek, *Int. J. Pept. Protein Res.*, 1983, **21**, 491.

Representations of Structures. An ellipsoidal approximation of protein structures has been developed by Taylor *et al.*[118] and by Prabhakaran.[119] Both papers suggest that the shape of a protein is characteristic of its tertiary structural class (*e.g.* all α, α/β, *etc.*). A slightly modified form of the 'helix wheel'[120] illustrates the relative orientations of residues in helices,[121] and a differential geometry representation has been developed to describe the path of a peptide chain in terms of its curvature and torsion.[122] This allows an objective definition of secondary structure, as does Kabsch and Sander's[74] pattern-recognition program mentioned earlier.

Protein Folding. Go and co-workers[123,124] have considered the randomness of protein folding using a two-dimensional lattice model, and Go[125] has presented a fuller review of the topic. Matheson and Scheraga[126] have used a Zimm–Bragg model for helix–coil transitions to demonstrate how the formation of hydrophobic pockets is important for helix nucleation. The importance of loop entropy on helix–coil transitions has been demonstrated by Skolnick[127] for two-chain coiled coils. A control study by Yuschok and Rose[128] shows that the hierarchic organization observed in real proteins can also be observed in simulated chain folds.

Models of Protein Dynamics. – Since the pioneering molecular-dynamics (MD) simulation of McCammon *et al.*,[129] technical advances have resulted in bovine pancreatic trypsin inhibitor (BPTI) simulations[4,129–131] that show improvements over preceding ones.[132–134] Levitt[4,130] claims that the two different hydrogen-bond potentials used in his recent MD simulations are more realistic than previously; both MD trajectories remain closer to the *X*-ray structures than earlier simulations. An analysis of one trajectory suggests that BPTI dynamics are *not* fluid-like and diffusional.[129] Rather, the protein atoms vibrate about one conformation before jumping into a new, slightly different conformation, suggesting that a solid-like harmonic description may be realistic. In addition to

[118] W. R. Taylor, J. M. Thornton, and W. G. Turnell, *J. Mol. Graphics*, 1983, **2**, 30.
[119] M. Prabhakaran, *Int. J. Pept. Protein Res.*, 1983, **22**, 371.
[120] M. Schiffer and A. B. Edmundson, *Biophys. J.*, 1967, **7**, 121.
[121] S. F. Abdulnur and K. Laki, *J. Theor. Biol.*, 1983, **104**, 599.
[122] A. H. Louie and R. L. Somorjai, *J. Mol. Biol.*, 1983, **168**, 143.
[123] N. Go and H. Abe, *Int. J. Pept. Protein Res.*, 1983, **22**, 622.
[124] N. Go and F. Kano, *Int. J. Pept. Protein Res.*, 1983, **22**, 633.
[125] N. Go, *Annu. Rev. Biophys. Bioeng.*, 1983, **12**, 183.
[126] R. R. Matheson, jun. and H. A. Scheraga, *Macromolecules*, 1983, **16**, 1037.
[127] J. Skolnick, *Macromolecules*, 1983, **16**, 1069.
[128] T. J. Yuschok and G. D. Rose, *Int. J. Pept. Protein Res.*, 1983, **21**, 479.
[129] J. A. McCammon, B. R. Gelin, and M. Karplus, *Nature (London)*, 1979, **267**, 585.
[130] M. Levitt, *J. Mol. Biol.*, 1983, **168**, 621.
[131] W. F. van Gunsteren, H. J. C. Berendsen, J. Hermans, W. G. J. Hol, and J. P. M. Postma, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 4325.
[132] M. Karplus and J. A. McCammon, *Nature (London)*, 1979, **277**, 578.
[133] W. F. van Gunsteren and M. Karplus, *Nature (London)*, 1981, **293**, 677.
[134] W. F. van Gunsteren and M. Karplus, *Biochemistry*, 1982, **21**, 2259.

the harmonic motions seen by Levitt, motions of a more collective character were seen in a simulation by Karplus *et al.*[135]

1983 saw the first attempts at a normal-mode description of BPTI. Go *et al.*[136] and Levitt *et al.*[137] allowed only torsion-angle variation and obtained broadly similar normal-mode frequency distributions ranging from 5 cm^{-1} to about 200 cm^{-1}. As there is MD evidence that fixing bond lengths and angles may halve root-mean-square (RMS) amplitudes,[138] Brooks and Karplus[139] included bond-length and -angle variation in a more sophisticated normal-mode analysis. Although their larger number of variables necessarily gave many more modes, the resulting frequency distribution in the range 6–200 cm^{-1} was similar in shape to those of Go and Levitt. Although RMS amplitudes showed trends similar to those from an MD simulation using the same potentials, the lowest-frequency modes were significantly anharmonic; large frequency increases and significant amplitude reductions resulted from recalculating these anharmonic frequencies. This finding, and the neglect of solvent, suggests that these modes are unlikely to be accurate. Thus we are not presently in a position to reproduce the essential dynamics of proteins using a small number of normal modes.

Reviews on general protein dynamics have been written by Karplus and McCammon[140,141] and by Abaturov *et al.*[142]

Theoretical Models of Protein Dynamics Applied to Experimental Observations. – Here we report dynamic models constructed to explain experimental observations and calculation of experimental quantities from MD simulations.

X-Ray Crystallography. The simulation of the BPTI unit cell by van Gunsteren *et al.*[131] included model solvent molecules. During their 20 ps run the four BPTI molecules wandered away from the *X*-ray structure and from each other; averaging over the four produced better positional agreement with the *X*-ray structure than in previous simulations. In contrast to Levitt,[4,130] van Gunsteren *et al.* did not explicitly include hydrogen bonding, as they claim it can be adequately modelled by electrostatic and van der Waals forces. The different treatment of the environment in these two simulations reflects different philosophies regarding potential functions. Levitt's simulation was *in vacuo*, and he argues that better agreement with *X*-ray data will come by improving potential functions in *in vacuo* simulations rather than by including an environment. In contrast, van Gunsteren *et al.* claim that their improved agreement with *X*-ray data is largely a result of their inclusion of an environment. Their

[135] M. Karplus, S. Swaminathan, T. Ichiye, and W. F. van Gunsteren in 'Mobility and Function in Proteins and Nucleic Acids', Ciba Found. Symp. 93, ed. R. Porter, M. O'Connor, and J. Whelan, Pitman, London, 1983, p. 271.
[136] N. Go, T. Noguti, and T. Nishikawa, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 3696.
[137] M. Levitt, C. Sander, and P. S. Stern, *Int. J. Quantum Chem.: Quantum Biol. Symp. 10*, 1983.
[138] W. F. van Gunsteren and M. Karplus, *Macromolecules*, 1982, **15**, 1528.
[139] B. Brooks and M. Karplus, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 6571.
[140] M. Karplus and J. A. McCammon, *Annu. Rev. Biochem.*, 1983, **53**, 263.
[141] J. A. McCammon and M. Karplus, *Acc. Chem. Res.*, 1983, **16**(6), 187.
[142] L. V. Abaturov, *Mol. Biol. (Moscow)*, 1983, **17**(4), 683.

success in reproducing the positions of crystallographic water was limited. The *R*-factor calculated using structure factors that were calculated from the MD run was 52%.

From an analysis of various types of atomic motion contributing to *X*-ray temperature factors, Gilbert *et al.*[143] conclude that atomic vibrations are the major component.

Mössbauer Spectroscopy. Mössbauer spectra of deoxymyoglobin exhibit a drastic change above a critical temperature, T_c. Below T_c Knapp *et al.*[144] and Bauminger *et al.*[145] agree that the Mössbauer spectrum is that of a typical solid-like harmonic haem environment and that the molecule is trapped in one conformational substate. Above T_c the dynamic model of Knapp *et al.* contains at least three Brownian oscillator modes, of which two are extremely overdamped and are postulated to characterize transitions between conformation substates. Bauminger *et al.* describe the probe motion above T_c in terms of fast fluctuations (including between conformational substates) and slower fluctuations that can be modelled equally well by a jump model for the iron or by overdamped Brownian oscillations. In a similar vein, Mayo *et al.*[146] have interpreted Mössbauer spectra of frozen solutions of deoxyhaemoglobin in terms of transitions between conformational substates.

Isotope Exchange. Sheridan *et al.*[147] performed a normal-mode analysis on the cyclic hexapeptide ferrochrome and calculated the resulting solvent exposure of the exchangeable groups as the molecule vibrates. Preliminary comparison with experiment shows that one of the protons that remains unexposed to solvent during the harmonic motion actually exchanges, implying that gross molecular unfolding may occur. Nagai *et al.*[148] have published further work on their random-walk model of polypeptide hydrogen exchange. They consider the boundary point between helix and coil regions of a polypeptide to behave as a weakly asymmetric random walker.

Nuclear Magnetic Resonance. Lipari *et al.*[149] have characterized carbon-13 n.m.r. relaxation in proteins partly in terms of a generalized order parameter, which is a measure of the angular restriction of the motion of a $^{13}C-H$ vector. The theoretical order parameters from a 96 ps simulation of BPTI by Karplus and McCammon[150] are larger than the experimental ones, indicating that there is less

[143] W. A. Gilbert, J. Kuriyan, G. Petsko, and D. Ringe Ponzi in 'Structure and Dynamics: Nucleic Acids and Proteins', ed. E. Clementi and R. Sarma, Adenine Press, New York, 1983, p. 405.
[144] E. W. Knapp, S. F. Fischer, and F. Parak, *J. Chem. Phys.*, 1983, **78**, 4701.
[145] E. R. Bauminger, S. G. Cohen, I. Nowik, S. Ofer, and J. Yariv, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 736.
[146] K. H. Mayo, D. Kucheida, F. Parak, and J. C. W. Chien, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 5294.
[147] R. P. Sheridan, R. M. Levy, and S. W. Englander, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 5569.
[148] Y. Nagai, M. Fujiwara, and N. Saito, *Biophys. Chem.*, 1982, **16**, 297.
[149] G. Lipari, A. Szabo, and R. M. Levy, *Nature (London)*, 1982, **300**, 197.
[150] M. Karplus and J. A. McCammon, *Nature (London)*, 1979, **277**, 578.

motional averaging in the simulation than measured experimentally, possibly owing to the length of the simulated trajectory being too short. Keniry *et al.*[151] and Schramm and Oldfield[152] have developed models for characterizing relaxation times in deuterium n.m.r. of proteins. Timofeev[153] contends that descriptions of the dynamic behaviour of protein side chains in terms of a single rotational correlation time are inadequate, preferring a model that includes a steric restriction parameter describing reorientation of the side chain and the rotational correlation time of the whole protein globule. Keniry *et al.*[151] have used a model that relates angular-dependent spin–lattice relaxation times in methyl-containing amino-acids to a three-fold jump rate for a methyl group hopping between equivalent sites. Villa *et al.*[154] showed theoretically that the mere presence of a solid surface causes the appearance of solid-like features in the n.m.r. response of the nearby water.

Fluorescence Depolarization. Ichiye and Karplus[155] used MD simulation of lysozyme to examine the picosecond fluorescence depolarization of tryptophan residues. Some highly mobile tryptophans had large fluorescence anisotropy decay whereas more rigidly confined tryptophans did not. They concluded that measurements employing short-time resolution (*e.g.* femtosecond laser techniques) would be of great value as a means of measuring picosecond amino acid side-chain dynamics in proteins.

Other Techniques. Low-frequency Raman peaks have been observed in several proteins between 20 cm^{-1} and 30 cm^{-1}.[156–158] Chou's continuity model of the α-helix (*i.e.* as a spring with distributed mass) yields fundamental frequencies that coincide with observed values.[159,160]

Bovine and human α-lactalbumins and carbonic anhydrase B can be transformed under certain influences into a state intermediate between the folded and unfolded states. A model of this intermediate state by Ptitsyn *et al.*[161] shows a substantial increase in the structural fluctuations and a sharp decrease in the van der Waals and other interactions. Likhtenshtein and Kotel'nikov[162] have analysed physical labelling-technique data and proposed a model of

[151] M. A. Keniry, R. L. Smith, H. S. Gutowsky, and E. Oldfield in 'Structure and Dynamics: Nucleic Acids and Proteins', ed. E. Clementi and R. Sarma, Adenine Press, New York, 1983, p. 435.
[152] S. Schramm and E. Oldfield, *Biochemistry*, 1983, **22**, 2908.
[153] V. P. Timofeev, *Mol. Biol. (Moscow)*, 1983, **17**, 519.
[154] M. Villa, L. Borghi, A. De Ambrosias, and S. Aldrovandi, *Nuovo Cimento Soc. Ital. Fis. D*, 1983, **2D**, 1019.
[155] T. Ichiye and M. Karplus, *Biochemistry*, 1983, **22**, 2884.
[156] K. G. Brown, S. C. Erfurth, E. W. Small, and W. L. L. Peticolas, *Proc. Natl. Acad. Sci. U.S.A.*, 1972, **69**, 1467.
[157] P. C. Painter, L. E. Mosher, and C. Rhoads, *Biopolymers*, 1982, **21**, 1469.
[158] L. Genzel, F. Keilman, T. P. Martin, G. Winterling, Y. Yacoby, H. Frohlich, and M. W. Maiken, *Biopolymers*, 1976, **15**, 219.
[159] K. C. Chou, *Biochem. J.*, 1983, **209**, 573.
[160] K. C. Chou, *Biochem. J.*, 1983, **215**, 465.
[161] O. B. Ptitsyn, D. A. Dolgikh, R. I. Gil'manshin, H. E. I. Shaknovich, and A. V. Finkel'hstein, *Mol. Biol. (Moscow)*, 1983, **17**, 569.
[162] G. I. Likhtenshtein and A. I. Kotel'nikov, *Mol. Biol. (Moscow)*, 1983, **17**, 505.

proteins as a system of tightly packed polypeptide blocks connected by hinges and with few degrees of freedom. The system is sunk in a viscous amorphous medium composed of water molecules and superficial protein groups. The use of e.p.r. saturation curves in studying biomolecular dynamics was analysed by Kulikov and Likhtenshtein.[163]

Protein Dynamics and Biological Function. – A central issue in bioenergetics is how energy released from ATP hydrolysis is transferred to where it is used. Davydov[164] has shown that energy transduction can take place *via* excited molecular vibrations. Although, according to linear analysis, energy transported by this means should spread out from the dispersive effects of dipole interactions, the non-linear character of interatomic forces in proteins may allow robust 'solitons' to carry energy over biologically significant distances. Scott's numerical model[165] of Davydov's theory shows that α-helix solitons may be expected under normal physiological conditions. He also develops an analytical theory for the internal vibrations of the soliton and presents agreement between soliton theory and laser scattering from metabolically active living cells. Jardetsky and King[166] have explained n.m.r.-observable motions in proteins in terms of rotational distortions of the protein backbone produced by the formation of solitons. Louie and Somorjai[122] have proposed that certain local structures may act as energy traps, leading to local excitations possibly near the active site.

Penta-ammineruthenium(III) (histidine-33)-ferricytochrome *c* contains Ru and Fe at 15 Å separation. At normal temperatures electron transfer between these two ions occurs unusually without the normal temperature dependence, in part, from polarization of the solvent. Freed[167] explains this lack of temperature dependence in terms of the influence of intramolecular vibrational relaxation on the electron-transfer rate.

Dutta-Roy and Bhaumik[168] have pointed out that macromolecules considered as open systems possessing low-frequency vibrational modes can produce the phenomenon of Bose condensation in the lowest-frequency mode. They discuss the functional consequences and suggest relevant experiments.

The role of solvent viscosity in protein dynamics and function has been investigated by Doster.[169] His model for dynamic friction assumes that the fluctuation spectrum has two components: solvent-independent diffusion of

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[164] A. S. Davydov in 'Structure and Dynamics: Nucleic Acids and Proteins', ed. E. Clementi and R. Sarma, Adenine Press, New York, 1983, p. 377.

[165] A. C. Scott in 'Structure and Dynamics: Nucleic Acids and Proteins', ed. E. Clementi and R. Sarma, Adenine Press, New York, 1983, p. 389.

[166] O. Jardetsky and R. King in 'Mobility and Function in Proteins and Nucleic Acids', Ciba Foundation Symposia 93, ed. R. Porter, M. O'Connor, and J. Whelan, Pitman, London, 1983, p. 291.

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[169] W. Doster, *Biophys. Chem.*, 1983, **17**, 97.

local structural defects in the protein matrix and global fluctuations coupled to the solvent. He applies the theory to the viscosity dependence of oxygen-binding rates in sperm-whale myoglobin, a subject that has been reviewed by Beece *et al.*[170]

Damjanovich *et al.*[171] have discussed kinetic and thermodynamic aspects of protein vibrations and enzyme–ligand interactions, paying special attention to the influence of electric fields and environmental microviscosity. The effect of immobilization on protein dynamics and activity has been investigated by Genkin *et al.*[172] with special reference to cytochrome *c* and to carbon monoxide binding to haemoglobin. Huber and Bennett[173] have studied the role of structural flexibility in protein function. In the trypsin : trypsinogen system an order–disorder transition is used to regulate enzyme activity. Immunoglobulins demonstrate how flexibility-linked domains may be used to allow the binding of ligands with diverse arrangements. In citrate synthase, domain motion forms an active site that is shielded from the solvent.

Several reviews concerning dynamics aspects of protein function appeared in 1983.[174–177]

2 Stability, Dynamics, and Folding of Proteins

Contributed by S. Craig

Stability. – Aspects of the folding, denaturation, and stability of proteins relevant to enzymes in industrial processes have been reviewed.[178] The stability of proteins that do not present a single co-operative transition is discussed.[179] An empirical approach to protein stability is outlined[180] along with its application to protein flexibility.

The apparent free-energy changes for the reversible processes between states *N*, I, II, and *D* and between II and *D* of lysozyme have been determined.[181] This allows estimation of the apparent free energy for transitions between any

[170] D. Beece, L. Eisenstein, H. Frauenfelder, D. Good, M. C. Marden, L. Reinisch, A. H. Reynolds, L. B. Sorendsen, and K. T. Yue in 'Hemoglobin Oxygen Binding', (Int. Symp. Interact. Iron Proteins Oxygen Electron Transp.), ed. C. Ho, Elsevier, New York, 1982, p. 363.

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[177] H. Frauenfelder in 'Structure and Dynamics: Nucleic Acids and Proteins', ed. E. Clementi and R. Sarma, Adenine Press, New York, 1983, p. 369.

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[181] F. Ahmed, C. C. Contaxis, and C. C. Bigelow, *J. Biol. Chem.*, 1983, **258**, 7960.

two of the conformations. Results presented[182] suggest that the unfolding of ribonuclease A by urea and GuHCl is a different process from that occurring with lithium salts. The renaturation of free and Sepharose-immobilized D-amino acid oxidase from 6M GuHCl has been studied.[183] A reversible helix–coil transition has been observed in the isolated rod domain of pig-stomach myosin with a T_m of 325 K,[184] and a direct link between crossbridge release and a helix–coil transition in myosin is reported.[185] A theory for coupling between the intramolecular helix–coil transition and an intermolecular nematic-isotope transition is described.[186] The thermal stability of the α-helical conformation in several structural regions of myosin rod has been investigated.[187]

A method of studying protein denaturation using a GuHCl gradient and high-performance gel permeation chromatography is described.[188] This method is used with a multi-dimensional spectroscopic measuring system to investigate the denaturation profiles of ribonuclease A, cytochrome *c*, and pepsinogen[189] and of a number of other proteins.[190,191] Results indicate that the process of protein denaturation is more complex than is normally accepted. The GuHCl denaturation of rabbit-muscle phosphorylase *b* has been studied,[192] and high sensitivity to denaturant is thought to be linked to its conformational flexibility.

The effects of guanidine and temperature on glucose dehydrogenase are described.[193] The results show that with increased temperature there is a decrease in α-helix content and increased β-sheet content, suggesting that the enzyme contains a stable core of β-sheet. Thermal denaturation studies have been carried out on native and crosslinked *B. cereus* β-lactamase I,[194] different forms of diphtheria toxin,[195] tropomyosin,[196] and α-mannosidase from cystic-fibrosis patients and carriers.[197] The thermal unfolding of detergent-solubilized and reconstituted cytochrome *b*5 has been investigated.[198] The thermostability of *Synechococcus* sp. ferredoxin may be due to special salt bridges.[199]

The conformational stability of type-1 collagen triple helix[200] and the effects of denaturants and ionic strength on the solution conformation of histone

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H1[201] have been studied. Conformational changes in bovine pancreatic trypsin inhibitor (BPTI) have been studied at atomic resolution.[202]

High-pressure effects on proteins are reviewed,[203] and the specific effects on the tertiary and quaternary structure of human-adult ferrous haemoglobin have been studied.[204]

Effects of Ligands. Normal and deuteriated phycosyanins have been studied.[205] The free energy of unfolding and the enthalpy are lower for the deuteriated proteins, which are also less resistant to urea than normal; however, the heat-capacity changes are similar. The binding of Mn^{2+} ions to beef-liver arginase appears to be important in stabilizing the enzyme along with a cluster of hydrophobic residues.[206] Collagen is found to have an increased thermal stability in the presence of sugars and polyols, and the stabilizing effects are similar to those that occur in globular proteins. This effect is due to the water-structure-making properties of sugars and polyols, which cause increased hydrophobic interaction in proteins owing to preferential solvation of the molecule.[207] General models are presented that can account for the existence of heat-capacity changes and compensation between the enthalpy and entropy changes in protein–ligand interactions.[208] The effects of the Hofmeister salts and urea series on acetylated and unmodified hexameric subunits of haemocyanin have been investigated,[209] and results suggest that polar and ionic interactions rather than hydrophobic forces stabilize the hexameric unit and the dodecamers in solution. The difference in thermal stability of foetal- and adult-brain *myo*-inositol 1-phosphate synthase in the temperature range 25–65 °C appears to be associated with the presence of an NAD cofactor.[210] The Na^+ and K^+ ion stabilization of the Na^+/K^+-dependent ATPase was accompanied by substantial increases in activation enthalpy and entropy, thus giving a less pronounced increase in activation free energy.[211] GTP has been found to stabilize proteolytic fragments of tubulin in an active conformation that allows them to assemble.[212] The order of effectiveness of substrates inhibiting reaction of the slowly reacting thiols of 3-phosphoglycerate kinase is very similar to the order of their protective effect against thermal inactivation.[213] Human choriogonadotropin is more resistant to thermal denaturation after 75% of its carbohydrate is removed.[214]

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Calcium-bound calmodulin undergoes a reversible conformation change at 22–28 °C; when high $[Ca^{2+}]$ are bound $T = 28$ °C, and when low $[Ca^{2+}]$ are bound $T = 22$ °C.[215] The effects of Ca^{2+} and Mg^{2+} binding on the thermostability of calmodulin and troponin C from skeletal muscle have been investigated,[216] and tryptic fragments have been used to investigate local structural changes. The conformation in alkali of native, acetylated, and guanidinated α-chymotrypsin has been investigated.[217] Guanidination increased the enzyme stability compared to the native form, whereas acetylation decreased the stability. In all cases removal of calcium decreased the amount of catalytically active conformation.

Effects of Individual Residues. The spatial clustering of amino acids as an evolutionary trend has been studied,[218] and structural conservation during protein evolution for classes of homologous proteins has been examined.[219] The conformational properties of pairs of amino acids are reported.[220] A 'working definition' of an ion pair is described,[221] and it appears that 76% of the ion pairs in a protein are involved in stabilizing tertiary structure. The terminal regions of a number of proteins appear to stabilize the tertiary or quaternary structure of the protein.[222]

A computer study has been used to investigate the stabilization of the right-handed twist of β-sheets by interchain interactions,[223] and the conformational rules governing coiling of β-pleated sheets are described.[224] The stabilizing effects of 2-methylalanine on β-turns and α-helices in peptides are described.[225] The left-handed $1\downarrow\beta 5,6$ helices of D,L alternating oligovaline in chloroform solution are found to be destabilized by increased chain length.[226] The primary sequences of two thermophilic and five mesophilic glyceraldehyde 3-phosphate dehydrogenases have been compared with the known three-dimensional structure of the enzyme in an effort to determine the factors responsible for thermal stability.[227] The results indicate tighter packing, increased hydrophobicity, and polarity of the S-loop region, which could increase subunit interaction. There are also indications that the helices are more stable in the thermophilic proteins. The acid denaturation of eleven cetacean myoglobins has been investigated to determine the contribution of individual amino acids to the stability of the

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molecule.[228] The results indicate that the conservation of stability is due to compensatory interactions. The single sulphydryl group of β-lactoglobulin has had propyl, aminoethyl, carboxyethyl, and hydroxyethyl groups attached by a disulphide bond prior to reversible urea denaturation.[229] The results suggest that the derivatives deviate from a two-state mechanism of unfolding.

The effects of *cis*- and *trans*-proline in Tyr-Pro and Pro-Tyr segments of tetrapeptides have been examined;[230] Pro appears to have a more pronounced effect on side-chain organization of residues preceding it than on those succeeding it in the sequence.

Solvent Effects. Aspects of the effects of water on folding, stability, and other properties of proteins are discussed.[231] Results reported[232] show water-induced, local, secondary structural perturbations that can be amplified in long helices. Reverse turns in proteins normally occur near the surface of a protein; however, buried turns have been discovered.[233] In all cases of buried turns, one or more of the main-chain atoms of the turn residues are hydrogen-bonded to the solvent, thus making the bound water molecules an integral part of protein structure. A simulation of water molecules in the intermolecular channels of [Phe^4-Val^6]-lanatomide dodecahydrate has been carried out to study peptide–water interactions.[234]

Terminal residues are found to destabilize the ordered structure of oligopeptides induced by sodium dodecyl sulphate (SDS).[235] Increased helical content for a number of protein–dodecyl sulphate complexes has been observed when the solution is acidified[236] as a result of increased helix propagation by glutamyl and aspartyl residues when protonated. A conformation change in cytochrome *c* has been observed on the binding of SDS and hexadecyltrimethylammonium chloride.[237]

Thermodynamic transfer functions have been used to rationalize the stabilizing effects of alcohols on protein and polypeptide conformation in aqueous solution.[238] The effect of 5% TCA on the denaturation of bovine pancreatic ribonuclease has been studied,[239] as has the preferential interaction of bovine serum albumin and lysozyme with concentrated solutions of glycine, betaine,

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and α- and β-alanine.[240] Acid-induced unfolding of Ca^{2+}- and Ca^{2+}–Mg^{2+}-specific domains of troponin C shows that both types are stabilized by protonation of carboxylate groups clustered in the binding loops.[241] The acid-induced $N \rightleftharpoons F$ transition of bovine plasma albumin may involve helix–β and helix–coil transitions.[242]

Dynamics of Proteins. – This field is reviewed,[243,244] and an overview of the problems in this area is given[245] in a review of two recent papers.[246,247] Mobility and function in proteins are described in a recently published book.[248]

The penetration of dioxygen[249] and small molecules[250] into proteins has been studied and the fluorescence depolarization of tryptophan residues in proteins investigated.[251]

The exchange-rate profile of buried amide protons has been shown to follow the calculated pH dependence of the electrostatic component of protein stability.[252] Hydrogen-exchange studies have been carried out on ferricytochrome,[253] phytochrome,[254] and lysozyme in crystal and solution.[255] The correlation of hydrogen-exchange behaviour with thermal stability of lysozyme is investigated.[256] Difference hydrogen-exchange studies have shown that lyophilized lysozyme does not have the same conformation as lysozyme in aqueous solution.[257]

The 132 picosecond simulation L79[246] has been analysed,[258] and a conceptual model has been proposed for protein dynamics in which a molecule vibrates about a particular conformation then suddenly changes to another conformation, jumping over an energy barrier. Calculated low-frequency vibrations of

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helical structures in α-chymotrypsin agree exactly with Raman data.[259] The peptide backbone of fd bacteriophase coat protein appears to be rigid but undergoes small-amplitude rapid motions.[260] The dynamics of the haem iron in met- and deoxy-myoglobin crystals are reported.[261]

A kinetic study of the dynamics of proton transfer between a surface moiety and bulk solvent is reported.[262] The nature and location of the slow-exchanging peptide amide protons in the region 1–19 of RNase S[263] and the exchange behaviour of the hydrogen-bonded amide protons in the helix formed by residues 3–13 have been investigated.[264] The pH and conformation dependences of amide-proton exchange of a defined-conformation cyclic dodecapeptide have been investigated.[265] The unfolding enthalpy of BPTI has a small dependence on temperature, and the resulting difference in dependence of Gibbs free energy of stabilization compared to other globular proteins makes the slow exchange rate of amide protons more understandable.[266] Internal motions in BPTI[267] and internal rotation in apo-haemoglobin α-chains[268] have been examined.

Supporting evidence for the existence of the 'molten-globule state' in proteins is given by results from the acid perturbation of cytochrome *c*.[269] In this state the polypeptide chain is packed as compactly as in the native state, but the side chains have high mobility. Conformation changes in insulin are transmitted over long distances.[270] The low internal compressibility of globular proteins is due to dynamic domains that are probably secondary structural elements.[271] The M13 coat protein has been reconstituted into phospholipid bilayers, and the mobility of the Phe and Tyr side chains has been investigated.[272]

The importance of flexibility for the functioning of immunoglobulins, trypsin-trypsinogen, and citrate synthase is described.[273] A range of snake neurotoxins has been studied,[274] and the order of irreversibility of neuromuscular blocking is the same as the order of structural rigidity of the toxins. A simulation of the thermal motions of atoms in ferrocytochrome *c* has been carried out, and the results are discussed in relation to structure and function.[275] The results of a

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simulation of a bond-breaking event in a fluctuating enzyme–substrate complex are discussed.[276] A normal-mode analysis has been performed on BPTI and results demonstrate this to be a useful method of examining internal motions of proteins.[277]

Stability and Association of Oligomeric Proteins. – The reconstitution of phosphoglycerate mutase from GuHCl[278] and the reassociation of the pyruvate dehydrogenase complex[279] have been studied. The relationship between stability and association equilibria is discussed.[280]

The folding and recognition of polypeptide chains on quaternary-structure formation are discussed.[281] The relationship of protein conformation to the kinetics of self-assembly of microtubules has been investigated.[282] In haemoglobin assembly electrostatic interactions are important determinants for correct assembly.[283] The effects of pH and Ca ions on the self-assembly of isolated subunits of *Limulus polyphemus* haemocyanin have been studied.[284]

Pepsin has been used to study conformational rearrangements in acid-dissociated lactic dehydrogenase.[285] This limited proteolysis has shown that the M1–M2 transition exposes a specific cleavage site thought to be Trp-225–Lys-226. A thermodynamic study of the assembly of *E. coli* aspartate transcarbamoylase is reported.[286]

Protein Folding. – *Intermediates in Protein Folding.* Two structural intermediates have been shown to exist on the folding–unfolding pathway of yeast iso-2-cytochrome *c*.[287] It is not known for one of the intermediates whether it is present at equilibrium in the unfolding transition or merely as a transient state. The other intermediate has a low time constant of about 10 s and is thought to be a highly condensed form of the protein. The use of D_2O and peptide —NH protons to trap intermediates in the folding of ribonuclease and the use of ^1H n.m.r. to investigate their structure have been described.[288]

The presence of state *H* as a stable structured state, intermediate in conformation between native and unfolded staphyloccocal β-lactamase, has been

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[285] G. Zettlmeissl, R. Rudolph, and R. Jaenicke, *Arch. Biochem. Biophys.*, 1983, **224**, 161.
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confirmed.[289] This intermediate contains native secondary structure but is more expanded than N and differs in tyrosine environment and in the environment of at least one of the two histidines present in the molecule.

The purification, characterization, and properties of intermediates in the folding of reduced hen egg-white lysozyme are reported.[290] Similar intermediates have been observed in the refolding of human and hen egg-white lysozymes, but the kinetics of appearance and disappearance of these intermediates and the native conformation are different for the two enzymes.[291] The difference in rate of refolding of hen egg-white lysozyme promoted by $Cu^{II} + O_2$ and by glutathione is thought to be due to the nature and/or amounts of intermediates on the pathway.[292]

The partially folded intermediates preceding the rate-limiting steps of refolding of BPTI have been investigated using circular dichroism to elucidate the conformational forces that determine a non-random, non-sequential pathway of disulphide-bond formation on refolding.[293] Further evidence of compact kinetic intermediates in the folding of the fragment complex of tuna cytochrome *c* is presented.[294] The dimeric intermediate on the reconstitution pathway of lactate dehydrogenase is found to be enzymatically active.[295] A common intermediate in the unfolding–refolding transition of *Streptomyces* subtilisin inhibitor is reported;[296] both transitions occur in two phases, although refolding is faster than unfolding. Kinetic intermediates in the folding of carbonic anhydrase are thought to be in the 'molten-globule state'.[297]

Refolding kinetics of reduced phospholipase A2 and cardiotoxin have been measured using h.p.l.c., and signals that may correspond to intermediate states have been observed.[298] Intermediates in the unfolding–refolding transition of T4 lysozyme have been observed,[299] and the non-coincidence of structural parameters in the unfolding–folding transition of horse-muscle phosphoglycerate kinase indicates the presence of equilibrium intermediates.[300]

The folding pathways of tail-spike protein mutant phage P22 do not show any accumulation of the partially folded protrimer intermediate as in wild type, although there is accumulation of an earlier intermediate in some mutants.[301]

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[299] M. Desmadril and J. M. Yon, *Biochemistry*, 1984, **23**, 11.
[300] M. Desmadril, A. Mitraki, J. M. Betton, and J. M. Yon, *Biochem. Biophys. Res. Commun.*, 1984, **118**, 416.
[301] D. P. Goldenberg, D. H. Smith, and J. King, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 7060.

Theoretical Studies. This field is reviewed.[302] Methods reported[303] can be used to produce a protein-folding algorithm, and using the fragment approach described a large protein can be treated. Restrained energy minimization and molecular dynamics are used to study protein folding.[304] The problem of minimum-energy pathway selection is examined in relation to topological aspects of conformational transformations occurring during folding.[305]

A two-dimensional lattice model is used to investigate the randomness of protein folding,[306] and results of the simulation indicate that events of folding–unfolding are stochastic rather than deterministic. These results are used to argue that a description of the pathway of folding should consist of a definition of intermediate states in terms of characteristic conformational features, together with stochastic rules of transitions between these intermediate states. Transitions occurring as a Markoffian process would be the simplest description, and this is examined.[307] The inclusion of native haem-protein contacts in the globule-coil model of folding of apomyoglobin changes the pathway from growth and merging of two native domains to growth of a single domain.[308]

Proline Isomerization. A proline-specific endopeptidase with an absolute specificity for a *trans* peptide bond is reported,[309] and this should prove a useful tool in the study of *cis–trans* isomerization rates in folding transitions. Trypsin is used to cleave specifically the peptide bond following the active bond in the *trans* state, and results from an investigation of oxidized RNase A suggest that overall chain dynamics may exert some effect on the kinetics of isomerization of proline.[310] Pro-93 in RNase A must be in the *cis* form before it can refold to the active conformation,[311] contradicting the possible existence of a native-like intermediate containing the incorrect *trans*-Pro-93 isomer on the refolding pathway, so that further results may be interpreted without assuming the appearance of true structural intermediates during base-line refolding.[312]

The class-I fructose 1,6-bisphosphate aldolase from *Staphylococcus aureus* has been studied, and the prolines present in its sequence appear to be non-essential as they do not limit the rate of folding.[313] Non-essential prolines have also been found in deer pancreatic ribonucleases.[314]

The intermediate and slow kinetic phases in the refolding of ferricytochrome *c* are due to proline isomerization, and the occurrence of the phases is dependent upon the conformation of the denatured protein and the ligation of Met-80 in the folded product.[315]

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[306] N. Go and H. Abe, *Int. J. Pept. Protein Res.*, 1983, **22**, 622.
[307] N. Go and H. Abe, *Int. J. Pept. Protein Res.*, 1983, **22**, 633.
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[314] H. Krebs, F. X. Schmid, and R. Jaenicke, *J. Mol. Biol.*, 1983, **169**, 619.
[315] D. N. Brems and E. Stellwagen, *J. Biol. Chem.*, 1983, **258**, 3655.

Simple, Sequential, and Hydrophobic Cluster Mechanisms. A simple model for the folding–unfolding transition kinetics of RNase A in urea has been proposed.[316] The model involves three independent structural mechanisms, a fast phase involving conformational changes and two rate-limiting slow phases that occur in the unfolded state. The slow phases are urea-insensitive chain equilibrations that do not involve conformational changes, one of them being caused by Pro-93 isomerization. Other results[317] argue against the 'simple mechanism' for RNase A, supporting instead the sequential-folding model *via* structural intermediates. A stable native-like structure is proposed as a refolding-pathway intermediate.

The refolding pathway of rabbit-muscle pyruvate kinase shows rapid formation of partially folded inactive molecules, some of which slowly rearrange to the active conformation.[318] The folding of Kringle 4 fragment[334] is also consistent with a pathway proceeding through subassemblies.

The unfolding of cobra neurotoxin and cardiotoxin consists of multi-state conformational transitions with a range of unstable transient states.[319] Histones H1 and H5 unfold reversibly in a two-state transition with large-scale side-chain motions preceding the co-operative transition.[320]

The periodicity of hydrophobicity in the amino acid sequence of a protein appears to be a factor affecting the formation of secondary structure, and in many sequences secondary-structure segments tend to form at a hydrophobic surface formed by other sections of the folded protein.[321] The chains in the non-helical domain of 59K short-chain cartilage collagen are thought to be tightly held by hydrophobic interactions, and on renaturation after heating they assist the rapid formation of the helical domain.[322]

Folding of Domains and Fragments of Proteins. The kinetics of recombination between S-peptide and S-protein in the refolding of RNase S have been measured.[323] The α-helix present in isolated S-peptide is shown to be limited to certain residues, some of which are helical in the intact protein, and a functional helix-termination signal exists in the isolated peptide.[324, 325]

Further evidence for the stepwise unfolding of the two domains of tryptophan synthase α-subunit is given.[326] Denaturation studies have shown a two-

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[326] H. Iwahashi, K. Yutani, K. Ogasahara, S. Tsunasawa, Y. Kyogoku, and Y. Sugino, *Biochim. Biophys. Acta*, 1983, **744**, 189.

domain structure for myoglobin[327] and antithrombin III,[328] both with multi-state transitions. Refolding studies of antithrombin III have shown that regions of the molecule do not return to a native conformation, although the Trp residues are all in the native environment.[329] The presence of twelve independently folding, compact, co-operative domains in the fibrinogen molecule is reported.[330]

The fragment 121–316 of thermolysin can refold into a stable native-like structure independent of the rest of the molecule,[331] as can fragment 206–316, which contains the whole of a domain structure.[332] The probable first step in unfolding of native thermolysin is separation of the two structural domains.[333] The structure of the Kringle 4 fragment of human plasminogen is thought to be an autonomous folding domain,[334] and results are consistent with a pathway folding through subassemblies. The conformation and folding of fragment 1–37 of thioredoxin have been studied.[335] The unfolding of aspartokinase homoserine dehydrogenase I is a stepwise process,[336] and results from the folding of fragments show that the kinase and dehydrogenase regions fold independently and that the native state is stabilized by tertiary interactions.[337] The 20K tryptic fragment of myosin S1 has shown some domain characteristics.[338]

Folding of Homologous Proteins. The folding of four different pancreatic ribonucleases shows that the pathway is conserved, extra prolines, substitutions, and carbohydrate chains not affecting the *in vitro* refolding pathway.[314] The refolding pathways of BPTI and venoms I and K from black mamba are very similar, showing evolutionary conservation of the pathway,[339] although the rates of interconversion of intermediates, the energetics of transitions, and the stability of the folded molecules differ. Circular and circularly permuted forms of BPTI have been made, and both can refold to the native conformation after reduction.[340]

The folding of chemically modified yeast iso-1-AM-cytochrome *c* is very similar to that of the native enzyme.[341] A Gly-211 to Glu substitution in the α-subunit of tryptophan synthase does not affect the secondary structure of the protein, but it halves the rate of refolding down to [urea] = 1M, below which it has the same rate as the native form and is urea independent.[342] The stability of

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[332] A. Fontana, C. Vita, and I. M. Chaiken, *Biopolymers*, 1983, **22**, 69.
[333] R. J. T. Corbett and R. S. Roche, *Biopolymers*, 1983, **22**, 113.
[334] M. Trexler and L. Patthy, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 2457.
[335] H. Reutimann, P. L. Luisi, and A. Holmgren, *Biopolymers*, 1983, **22**, 107.
[336] K. Muller and J. R. Garel, *Biochemistry*, 1984, **23**, 651.
[337] K. Muller and J. R. Garel, *Biochemistry*, 1984, **23**, 655.
[338] A. Muhlrad and M. F. Morales, *Proc. Natl. Acad. Sci. U.S.A.*, 1984, **81**, 1003.
[339] M. Hollecker and T. E. Creighron, *J. Mol. Biol.*, 1983, **168**, 409.
[340] D. P. Goldenberg and T. E. Creighton, *J. Mol. Biol.*, 1983, **165**, 407.
[341] E. H. Zuniga and B. I. Nall, *Biochemistry*, 1983, **22**, 1430.
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tryptophan synthase α-subunit from *E. coli*, *Salmonella typhimurium*, and an interspecies hybrid has been examined.[343]

Ovalbumin synthesized *in vitro* is found to have different properties from those of the renatured '*in vivo*' molecule.[344] Mutants of the phage P22 tail-spike protein have been studied,[345] and they show that the *in vivo* folding pathway is in two parts. The early part is sensitive to increased temperature, whereas the second part is inhibited by low temperature. It is also suggested that the pathway is controlled by the interactions non-essential for stability of the native state.

3 Infrared and Raman Spectroscopy

Contributed by R. M. Stephens

Model Compounds. – The frequencies of intermolecular modes in non-deuteriated and fully deuteriated α-glycine have been measured at 300 and 85 K by Raman and i.r. scattering techniques. The frequencies were analysed in terms of simple analytic interaction potentials. Buckingham potentials were assumed for the non-bonded and hydrogen-bond interactions, and Coulomb and screened Coulomb potentials were assumed for the electrostatic interactions. The observed frequencies are well described by the simple model, and the parameters of the hydrogen-bond potentials and the molecular-charge distribution were determined from the analysis.[346] The Raman spectra of zwitterionic glycylglycine crystal and its *N*-deuteriated analogue as well as a normal-co-ordinate analysis of its α-crystalline form have been analysed, and the effect of intra- and inter-molecular couplings has been discussed. A modified Urey–Bradley potential was used as a model of the intramolecular force field. Factor-group splittings were described by the use of intermolecular potentials consisting of non-bonded atom–atom and dipole–dipole interactions. Effects of hydrogen bonds on the vibrational frequencies of amino and carboxylate groups were also analysed.[347] A similar analysis was performed on glycyl-L-proline. Cyclic redundancies were eliminated, and those due to the ring methylene groups, particularly distorted in regard to the tetrahedral conformation, were treated. An assignment of the experimental frequencies was proposed on the basis of the normal-co-ordinate analysis.[348]

Far-i.r. spectra between 20 and 600 cm^{-1} have been obtained for L-alanine at temperatures between 6 and 300 K. Understanding of the temperature-sensitive

[343] K. Yutani, T. Sato, K. Ogasahara, and E. W. Miles, *Arch. Biochem. Biophys.*, 1984, **229**, 448.

[344] R. D. Klausner, J. N. Weinstein, R. Blumenthal, and J. Van Renswoude, *Biochem. J.*, 1983, **212**, 801.

[345] D. P. Goldenberg, D. H. Smith, and J. King, *Biopolymers*, 1983, **22**, 125.

[346] B. Andrews, B. H. Torrie, and B. M. Powell, *Biophys. J.*, 1983, **41**, 293.

[347] P. Lagant, G. Vergoten, M. H. Loucheux-Lefebvre, and G. Fleury, *Biopolymers*, 1983, **22**, 1267.

[348] P. Lagant, M. H. Loucheux-Lefebvre, J. P. Huvenne, G. Vergoten, G. Fleury, and P. Legrand, *Biopolymers*, 1983, **22**, 1285.

as well as the temperature-insensitive bands was aided by the normal-co-ordinate analysis of L-alanine. Bands containing contributions from hydrogen bonds, skeletal deformation, and NH_3^+ torsion were highly sensitive to temperature changes. Although the hydrogen bonds were stronger at lower temperatures, no evidence of amide formation was detected even at 6 K. Thus the zwitterion form seems to be more stable than the amide form in the temperature range studied.[349] A phase transition has been observed at 80 °C in crystalline L-leucine, and the heat of transition was estimated to be 170 J mol^{-1} by d.s.c. measurement. The analysis of the i.r. and Raman spectra and MNDO conformational analyses show that the transition is purely a conformational type without a disorder effect, mostly localized in the alkyl side chain and nearly without effect on the double-layer structure.[350]

The preferred conformations of $Me_3CO_2CAla_5(G^1\text{–}G^2)Ala_3NHPEGM$, where G^1 and G^2 are Glu(OCH_2Ph) and Lys(CO_2CH_2Ph), respectively, were studied in solvents of different polarity and in the solid state using c.d. and i.r. absorption techniques. The Ala_{10} homopeptide adopted a pure β-structure, but the insertion of the two residues G^1 and G^2 resulted in partial or total disruption of the ordered conformation. The extent of β-structure was determined as a function of the CH_2Cl_2/DMF ratio by monitoring the intensity of the 1630 cm^{-1} i.r. band.[351] The far-i.r. spectrum of DL-phenylalanine was recorded on an FIR-30 spectrometer with Raman spectra of the same molecule recorded on a CODERG T800 spectrometer, using the 514 nm exciting line. Various fundamentals obtained in far-i.r. and laser Raman spectra were correlated and assigned to particular normal modes of vibration.[352]

Vibrational studies of a variety of mureins and some of their components showed that (1) the peptide moiety of this macromolecule is non-crystalline and there are no regular structures such as helices of β-sheets, but its conformation is not completely unordered (random like), and (2) the spectral characteristics of the synthetic peptides L-Ala-D-Glu-α-L-Lys-D-Ala-D-Ala and D-Glu-α-L-Lys-D-Ala-D-Ala were very different from those of natural peptidoglycans, whereas corresponding features of natural murein and the synthetic peptide MurNAc-L-Ala-D-Glu-α-L-Lys-D-Ala were very similar. Among the natural peptidoglycans surveyed were those from *Staphylococcus aureus*, *Micrococcus lutens*, and *Bacillus subtilis*. It was concluded that there exist specific non-covalent-bonding interactions between the sugar and the peptide part of native murein that may lead to reverse-turn types of arrangements.[353]

Fourier-transform i.r. and resonance Raman studies together with *X*-ray crystallography data were used to define the three major conformational states

[349] J. Bandekar, L. Genzel, F. Kremer, and L. Santo, *Spectrochim. Acta, Part A*, 1983, **39**, 357.

[350] D. Bougeard, *Ber. Bunsenges. Phys. Chem.*, 1983, **87**, 279.

[351] M. Mutter, F. Maser, B. Klein, C. Toniolo, and G. M. Bonora, *Pept. Proc. Eur. Pept. Symp. 17th*, 1983, 729.

[352] V. Kumar and P. K. Verma, *Indian J. Pure Appl. Phys.*, 1983, **21**, 59.

[353] D. Naumann, E. Fischer, W. Roenspeck, G. Barnickel, H. Labischinski, and H. Braduczek, *Target Penicillin, Murein Sacculus Bact. Cell Walls Architect. Growth Proc. Int. FEMS Symp.*, 1983, 78.

of *N*-acylglycine dithioesters in solution and to set up spectra–structure correlations. The resonance Raman spectra were used to follow conformational events within dithioester enzyme–substrate intermediates. Further characterization was obtained from the synthesis and subsequent analysis of ^{13}C and ^{15}N derivatives of *N*-benzoylglycine ethyl dithioester and *N*-(β-phenylpropionyl)glycine ethyl dithioester. The observed isotope shifts offer insight into the normal-mode character of the resonance Raman bands and provide standards with which to compare the shifts in the corresponding enzyme–substrate intermediates.[354]

Polypeptides. – A series of poly(L-leucine) peptides, with average d.p. in the range between 10 and 100, and various poly(DL-leucine) peptides were prepared by polymerization of the corresponding carboxyanhydrides. The Fourier-transform (FT) i.r. spectra show that the amide I and II bands are exclusively sensitive to the secondary structure of the poly(leucine)s and not to their tacticity. The tacticities of the poly(DL-leucine)s were determined in CF_3CO_2H by ^{13}C n.m.r., whereas ^{13}C n.m.r. CP/MAS spectra of the solid polymers revealed that the poly(DL-leucine)s contain 40–65% helical structure. Consequently the broad amide I and II bands do not enable one to distinguish between isotactic and more or less atactic poly(leucine)s. However, the i.r. bands between 500 and 900 cm^{-1} allow a differentiation between poly(DL-leucine)s of different tacticity. The i.r. spectra confirmed that primary-amine-initiated polymerization of DL-leucine *N*-carboxyanhydrides does not lead to long stereo blocks.[355]

Conformational-energy computations were carried out for parallel and antiparallel β-sheets composed of poly(L-valine) (I) and poly(L-isoleucine) (II) peptide chains, each consisting of four and of six residues, respectively, with MeCO— and —NHMe end groups. The β-sheets considered contained three and five equivalent chains, respectively. All computed minimum-energy β-sheets had a large right-handed twist of a magnitude that corresponded to the mean twist of β-sheets observed in globular proteins. The twist had the same sign but was much larger than in β-sheets of poly(L-alanine) (III) because of intra- and interchain interactions between the bulky β-branched side chains. Whereas the right-handed twist was a result of intrachain interactions between side chains in the case of I, these interactions would favour a left-handed twist in II, and the right-handed twist in the latter was a result of interchain interactions. Parallel sheets were more stable than antiparallel sheets for both I and II, in contrast to III. This agreed with observations on the preferred orientation of the chains in oligopeptides that form β-structures. It also explained the observed high relative frequencies of occurrence of valine and isoleucine residues in parallel β-sheets, as compared with antiparallel sheets, in globular proteins.[356]

Proteins. – *Bacteriorhodopsin.* The bacteriorhodopsin photocycle consists of a series of intermediates, each characterized by a different visible absorption spectrum. One of these intermediates, L550, has been examined by analysing

[354] H. Lee, A. C. Storer, and P. R. Carey, *Biochemistry*, 1983, **22**, 4781.
[355] J. Stulz, D. Mueller, W. E. Hull, and H. R. Kricheldorf, *Makromol. Chem.*, 1983, **184**, 1311.
[356] C. K. Chou, G. Némethy, and H. A. Scheraga, *J. Mol. Biol.*, 1983, **168**, 389.

the Raman spectra of purple membrane recorded under different conditions. A rotating cell was used to control the contribution of the early intermediates in the spectra. These spectra were then fitted to a superpositon of Voigtian line-shapes to obtain the position, width, and integrated intensity of each band. Evidence was obtained for the appearance within 5 ms of two bands at 1539 and 1551 cm^{-1}. By correlating the intensity changes in these bands with the protonated Schiff-base band, it was established that the intermediates giving rise to them were protonated. Thus M412 is the first deprotonated intermediate in the bacteriorhodopsin photocycle. Because the ratio of the integrated intensity of the 1539 and 1551 cm^{-1} bands remains constant for various exposure times and laser power, the possibility that they both arise from the L550 intermediate rather than from the L550 and a second intermediate is discussed.[357]

The excitation profiles for twelve vibrational modes of light-adapted bacteriorhodopsin using excitation wavelengths from 472 to 752 nm were shown to be quite similar and have approximately the same shape and width as the absorption spectrum. Calculations using a 29-mode harmonic sum over states with large amounts of inhomogeneous broadening gave good fits to the absorption- and excitation-profile band shapes and to the absolute absorption and Raman cross-sections. These calculations also gave the intensities of the overtone and combination bands. The upper limit of 250 cm^{-1} for the homogeneous linewidths, as well as the absence of strong low-frequency Raman lines, implies that the lack of resolution in the bacteriorhodopsin absorption is due primarily to inhomogeneous effects.[358]

By measuring the rhodopsin–bathorhodopsin, rhodopsin–metarhodopsin II, isorhodopsin–bathorhodopsin, and rhodopsin–isorhodopsin difference spectra by FT i.r. spectroscopy, the C=N stretching vibration of the protonated retinylidene Schiff base of rhodopsin, isorhodopsin, and bathorhodopsin was identified. In contrast to resonance Raman spectroscopy, additional strong bands were observed between 1700 and 1620 cm^{-1}. Most of them depend on the isomeric state of the chromophore, and the origins of these bands were discussed, but no conclusions could be drawn on the isomeric state of retinalin bathorhodopsin.[359]

Spectra of the θ_{640} photointermediate of bacteriorhodopsin were obtained using a dual-beam, time-resolved technique, and a flowing purple-membrane suspension was illuminated with a 514 nm laser beam to initiate photocycling. Raman scattering from θ_{640} was excited 3–4 ms downstream with a probe beam at 752 nm. Raman spectra of the θ_{640} intermediate were obtained from native material in H_2O and D_2O as well as from material regenerated with 15-deuterio- and 12,14-dideuterio-retinals. θ_{640} had a Schiff-base line at 1628 cm^{-1} that shifted to 1589 cm^{-1} in D_2O, demonstrating that the base is protonated. The pattern of vibrations in the 1100–1400 cm^{-1} region of the θ_{640} spectrum was similar to that observed in bacteriorhodopsin I_{568}, which contains an all-*trans* protonated Schiff-base chromophore. The changes observed upon deuteriation of the chromophore in I_{568}, the 13-*cis* component of dark-adapted I, were

[357] P. V. Argade and K. J. Rothschild, *Biochemistry*, 1983, **22**, 3460.
[358] A. B. Myers, R. A. Harris, and R. A. Mathies, *J. Chem. Phys.*, 1983, **79**, 603.
[359] F. Siebert, W. Maentele, and K. Gerwert, *Eur. J. Biochem.*, 1983, **136**, 119.

quite different from those observed in θ_{640}. This demonstrated that the retinal chromophore θ_{640} had an all-*trans* configuration. Consequently the M_{412}/θ_{640} conversion involved 13-*cis* all-*trans* chromophore isomerization and protonation of the retinal-lysine Schiff base.[360] Further reports on time-resolved spectra of the bathorhodopsin photocycle are to be found in references 361–364.

Carboxypeptidase A. Low-temperature multi-channel resonance Raman spectroscopy was used to ascertain the extent to which acyl-enzyme intermediates accumulate in the carboxypeptidase A-catalysed hydrolysis of ester substrates. Previous investigations suggested that active-site residue glutamate-270 acts as a nucleophile forming a transient enzyme-bound mixed-anhydride intermediate. A resonance Raman study of carboxypeptidase A-catalysed hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyl acetate and *O*-(*trans*-*p*-dimethylaminocinnamoyl)-L-β-phenyl acetate at sub-zero temperatures in aqueous organic solvents showed no accumulation of enzyme-bound mixed-anhydride intermediates.[365]

Carotenoproteins. The resonance Raman spectra of spirilloxanthin from *R. rubrum* showed two bands in the C=C stretching region. The band at 1508 cm^{-1} was independent of the wavelength of the excitation line and was attributed to the all-*trans* configuration. The other broad band, the frequency of which varied with excitation wavelength, was attributed to an assembly of heterogeneous *cis* configurations of the carotenoid. The configurations in the carotenoproteins that give rise to the two bands were considered to be stabilized by interactions with the apopeptide. The band at 1543 cm^{-1} appeared and increased in intensity relative to the 1508 cm^{-1} band during treatment of *R. rubrum* chromatophores with SDS and β-mercaptoethanol. Thus the *cis* configurations in the carotenoproteins are ascribable to the isomerization of the all-*trans* carotenoid in the solubilizing procedure.[366]

Chymotrypsin. The imidazole derivative of chromophoric *p*-dimethylaminobenzoic acid reacts with chymotrypsin in the pH range 4–7 to form a stable acyl intermediate that gave very good resonance Raman spectra. The spectra of the acyl-enzyme intermediate were compared with the spectra of simple model compounds, such as the corresponding methyl ester, aldehyde, and imidazole. The model compounds exhibited a linear correlation between the maximum wavelength of absorption and the frequency of the carbonyl vibration. The results showed that the chromophoric serine ester of the acyl-enzyme behaves

[360] S. O. Smith, J. A. Pardoen, P. J. Mulder, B. Curry, J. Lugtenburg, and R. Mathies, *Biochemistry*, 1983, **22**, 6141.
[361] C. L. Hsieh, M. A. El-Sayed, M. Nicol, M. Nagumo, and J. H. Lee, *Photochem. Photobiol.*, 1983, **38**, 83.
[362] S. O. Smith, M. Braiman, and R. Mathies, *Time-Resolved Vib. Spectrosc. (Proc. Int. Conf. TRVS)*, 1983, 219.
[363] T. Alshuth, I. Grieger, and M. Stockburger, *Time-Resolved Vib. Spectrosc. (Proc. Int. Conf. TRVS)*, 1983, 231.
[364] A. Lewis, *Time-Resolved Vib. Spectrosc. (Proc. Int. Conf. TRVS)*, 1983, 239.
[365] S. J. Hoffman, S. T. Chu, H. Lee, E. T. Kaiser, and P. R. Carey, *J. Am. Chem. Soc.*, 1983, **105**, 6971.
[366] M. Kito, J. Yamashita, and Y. Koyama, *Photobiochem. Photobiophys.*, 1983, **5**, 209.

differently from an ordinary ester. Thermal unfolding of the acyl-enzyme produces spectroscopic parameters close to those of the model ester. The carbonyl frequencies of a series of *para*-substituted benzoyl methyl esters showed a linear correlation with the rate of deacylation of the corresponding acyl-enzymes.[367]

5-Methylthienylacryloyl-chymotrypsin was prepared with ^{13}C. The labelled species confirmed the assignments of several of the bands in the resonance Raman spectrum of the acyl-enzyme and showed that the feature occurring near 1260 cm^{-1} involves the motion of the carbonyl carbon atom. It is likely that the 1260 cm^{-1} band is associated with the stretching vibration of the C—O bond being cleaved in the active site. A moderately intense 1260 cm^{-1} feature occurred only in the resonance Raman spectrum of the native acyl-enzyme, and possible reasons were given for its appearance. The labelled species also confirmed that the bands appearing near 1715 cm^{-1} in the spectrum of the acyl-enzyme were associated with the stretching mode of the acyl carbonyl.[368]

Cytochromes. Stabilized intermediate redox states of cytochrome *c* are generated by radiolytic reduction of initially oxidized enzyme in glass matrices at liquid-nitrogen temperatures. In the intermediate states the haem group is reduced by hydrated electrons, whereas the protein conformation is restrained close to its oxidized form by the low-temperature glass matrix. The intermediate and stable redox states of cytochrome *c* at neutral and alkaline pH are studied by low-temperature resonance Raman spectroscopy using excitations in resonance with the B (Soret) and Q (β) optical transitions. The observed spectral characteristics of the intermediate states, as well as of the alkaline transition in the oxidized state, are interpreted in terms of oxidation-state marker modes, spin-state marker modes, haem-Fe-axial-ligand stretching modes, totally symmetrical in-plane modes, non-totally symmetrical in-plane modes, and out-of-plane modes.[369]

The Fe–histidine stretching Raman line of the high-spin a_3 haem of the aa_3-type cytochrome oxidases has been assigned for mammalian yeast and bacterial enzymes in the reduced state. The isotope replacement of the haem Fe of the bacterial enzyme was worked out by cultivating a thermophilic bacterium in a ^{56}Fe- or ^{54}Fe-enriched synthetic medium. The Raman line of the reduced bacterial cytochrome oxidase at 210 cm^{-1} exhibited an upward frequency shift of 2 cm^{-1} on ^{54}Fe substitution, whereas other lines remained unshifted. This Raman line disappeared on binding of CN^- to the sixth co-ordination position. On raising the pH, the 210 cm^{-1} line lost intensity without any frequency shift. All of these features, which were consistent with the characteristics of the Fe–histidine stretching Raman lines of horseradish ferroperoxidase, deoxyhaemoglobin, and deoxymyoglobin, strongly suggested that the 210 cm^{-1} line of the bacterial cytochrome oxidase arises primarily from the Fe–histidine stretching vibration of the high-spin a_3 haem. However, the Raman spectral patterns of

[367] P. V. Argade, G. K. Gerke, J. P. Weber, and W. L. Petricolas, *Biochemistry*, 1984, **23**, 299.
[368] P. R. Carey and D. J. Phelps, *Can. J. Chem.*, 1983, **61**, 2590.
[369] B. Cartling, *Biophys. J.*, 1983, **43**, 191.

bovine-heart and yeast cytochrome oxidases were somewhat different from that of the bacterial enzyme.[370]

The P-450 active site of cytochrome has been studied. The difference spectra of oxidized P-450–substrate complex indicate a $-4.9\ cm^{-1}$ downshift in the case of ^{34}S isotopic substitution and a $+2.5\ cm^{-1}$ upshift in the case of ^{54}Fe isotopic substitution. This demonstrates the existence of an Fe—S linkage in the oxidized complex. Further, the $351\ cm^{-1}$ mode is assigned to the predominantly Fe—S stretching vibration.

Theoretical predictions of the ^{54}Fe–^{54}Fe and ^{32}S–^{34}S Raman shifts were made by using a three-body oscillator model. The S shift was strongly dependent on the C—S—Fe bond angle, whereas the Fe shift was not.[371] A detailed quantitative evaluation is reported on Franck–Condon and vibrational coupling effects on the excitation profile of the $1362\ cm^{-1}$ fundamental of cytochrome *c* in the Soret-band region. The asymmetry of this profile is caused by interference between both effects. A new approximation method is introduced to evaluate multi-mode Franck–Condon contributions to Raman scattering. Accurate values are derived for several properties of the B state corresponding to the Soret band.[372]

Fibrin. Spectroscopic studies of fibrin and fibrinogen have indicated a large increase in the β-sheet structure of fibrin during polymerization. The changes were from 8% in fibrinogen to a plateau of ~25% at the end of the polymerization process. This increase in β-sheet structure was favoured by conditions such as low ionic strength and low protein concentration. It was suggested that the changes in conformation were due to the formation of a large number of hydrogen bonds perpendicular to the fibre axis. These bonds were apparently formed by interaction of C-terminal regions of the α-chain of the D domain.[373]

Flavin. The resonance-coherent anti-stokes Raman scattering spectra for a number of flavoproteins are found to be fingerprints for the particular type of flavoprotein. One group studied were the bacterial flavodoxins. Other examples included the enzymes lactate mono-oxygenase and glucose oxidase, FMN complexed to *Vibrio harveyi* luciferase, and a partially characterized non-fluorescent flavoprotein from *Photobacterium leiognathi.* In the frequency range 1200–$1100\ cm^{-1}$, differences in the position and intensity of the prominent bands were reflections on the interactions of the isoalloxazine ring with the protein. The spectra were interpreted in terms of hydrogen-bonding between the amino acid residues of the binding site and particular atoms of the isoalloxazine ring.[374]

[370] T. Ogura, K. Honnani, T. Oshima, S. Yoshikawa, and T. Kitagawa, *J. Am. Chem. Soc.*, 1983, **105**, 7781.

[371] P. M. Champion, B. R. Stallard, G. C. Wagner, and I. C. Gunsolus, *Dev. Biochem.*, 1982, **23**, 547.

[372] W. Siebrand and M. Z. Zgierski, *Chem. Phys.*, 1983, **77**, 35.

[373] G. Hudry-Clergeon, J. Marx, and M. Berjot, *Fibrinogen Struct. Funct. Aspects Metab. Proc. Workshop*, 1983, 37.

[374] A. J. Visser, J. Vervoort, D. J. O'Kane, J. Lee, and L. A. Carreira, *Eur. J. Biochem.*, 1983, **131**, 639.

The resonance Raman spectra of the purple intermediates of D-amino acid oxidase reconstituted with isotopically labelled FAD's have been compared with those of the native enzyme. The lines at 1605 and 1548 cm^{-1}, with D-alanine or D-proline as substrate, underwent isotopic shifts upon 4a-^{13}C and 4,10a-$^{13}C_2$ labelling. These lines were assigned to the vibrational modes associated with the C_{10a}]:C_{4a} moiety of reduced flavin, providing the first assignment of resonance Raman lines of reduced flavin and conclusive evidence that reduced flavin is involved in this intermediate.[375]

Gramicidin. The orientation of gramicidin A incorporated in dimyristoylphosphatidyl choline vesicles has been examined using polarized i.r. spectroscopy. The band frequencies indicated that the gramicidin had a single-stranded π_{LD} structure, and the band dichroisms implied a perpendicular orientation of the gramicidin transmembrane channel with the π_{LD} structure, with the π_{LD} helix axis at approximately 15–20° with respect to the normal to the bilayer.[376]

Haemoglobin. Resonance Raman spectra of haem *a* complexes containing Fe^{II} and Fe^{III} in low- and high-spin states have been assigned by comparison with photohaem analogues and by deuteriation of the formyl substituent. Specific effects of the *trans*-conjugated formyl and vinyl groups included (1) resonance Raman enhancement of formyl and vinyl modes, (2) *x*,*y* splitting of the Q absorption bands by 1600 cm^{-1}, (3) depolarization ratios near one third for most resonance Raman bands, (4) resonance Raman activation and splitting of E_u skeletal modes with selective enhancemensts of the *x*- and *y*-polarized components, and (5) skeletal-mode shifts associated with the stabilization of one of the alternative 18-membered delocalization pathways of the porphyrin II system. For $(ImH)_2Fe^{II}Pa$ (ImH = imidazole, Pa = porphyrin *a*) increased Q-band splitting (~2000 cm^{-1}) and skeletal-mode shifts are attributed to $d_\pi \rightarrow$ porphyrin π^* back-bonding. For all complexes the C=O frequency is lower in aqueous than in non-aqueous solutions, owing to formyl hydrogen-bonding. The skeletal frequencies above 1450 cm^{-1} correlate with porphyrin core size, as they do for protohaem complexes, and with the same slopes.

The 413 nm resonance Raman spectrum of oxidized cytochrome oxidase is dominated by haem a_3 whereas the 454 nm spectrum of the reduced protein is dominated by the haem *a*. In the oxidized form, the low-frequency resonance Raman spectrum is interpreted in terms of haem a_3 perturbed by protein-peripheral substituent interactions, instead of Cu^{2+} vibrations as recently suggested. The haem a_3 skeletal frequencies are consistent with e.x.a.f.s. results, indicating a long bond to the sixth ligand.[377]

Reasonance Raman spectra of the M Boston [His-E7(58)$\alpha \rightarrow$Tyr] (I), Hb M Iwate [His-F8(87)$\alpha \rightarrow$Tyr] (II), and Hb M Milwaukee [Val-E11(67)$\beta \rightarrow$Glu] (III) showed that the abnormal subunits of I and II exhibited the porphyrin ν/10 bands at 1628 and 1627 cm^{-1}, respectively, which indicates that the

[375] R. Miura, Y. Nishina, M. Ohto, H. Tojo, K. Shiga, H. Watari, T. Yamano, and Y. Miyake, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 588.
[376] E. Nabedryk and J. Breton, *Stud. Phys. Theor. Chem.*, 1983, **24**, 391.
[377] S. Choi, J. Lee, Y. H. Wei, and T. G. Spiro, *J. Am. Chem. Soc.*, 1983, **105**, 3692.

ferric α-haems are five-co-ordinated in both I and II. In addition to the porphyrin bands four extra polarized lines were observed at 1607, 1506, 1278, and 603 cm^{-1} for the subunit of I and at 1605, 1506, 1310, and 589 cm^{-1} for that of II. By comparison with the vibrational spectra of Fe-tyrosine proteins and Fe-phenolate complexes, the 1605–1607 and 1506 cm^{-1} lines are assigned to the phenolate ring vibrations of the haem-co-ordinated tyrosine and to the phenolate CO stretching mode. It was proposed that the 603 cm^{-1} line of I and the 589 cm^{-1} line of II arise from the Fe—O (tyrosine) stretching mode. These four Raman lines are intensity-enhanced on excitation at approximately 475–520 nm, probably owing to the presence of a charge-transfer interaction between Fe and tyrosine. The dissimilarity of the Fe—O and phenolate CO stretching frequencies between I and II, despite the similarity of the frequencies of their porphyrin and phenolate ring modes, suggests that the haem–phenolate bonding angles differ between I and II, although both adopt the five-co-ordinate form, with tyrosine as the only axial ligand.[378] The quaternary-structure dependence of the haem vibrational modes in ligated haemoglobins indicates that haem–globin interactions in the distal haem pocket are largely invariant to quaternary structure perturbations.[379]

Second-derivative FT i.r. spectra of the proteins RNAse A, haemoglobin, and β-lactoglobulin A have been obtained in deuterium oxide solution from 1350 to 1800 cm^{-1}. In the second-derivative spectra, clearly resolved peaks are observed that can be associated with the α-helix, β-strands, and turns. Protein spectra with such resolution have not been reported before. Tentative assignments are proposed, and the observed peaks are related to the secondary structure of the proteins. The data may present the first direct spectroscopic evidence of turns in a native protein.[380]

The pH dependence of the oxidation-state marker line of haemoproteins was investigated in cytochrome *c* peroxidase with Raman difference spectroscopy. The frequency is sensitive to ionization of a group on the protein that regulates catalytic activity of the resting ferrihaem enzyme. The oxidation-state marker line shows a transition with p*K* of 5.5 in good agreement with other spectroscopic and kinetic measurements of binding of peroxide and other ligands to the native enzyme. The shift of 0.8 cm^{-1} to higher frequency at pH 4.5 relative to the pH 6.4 value is interpreted in terms of a substantial decrease in π-electron delocalization in the porphyrin ring. Charge delocalization in the π-system is highest at maximal activity, as would be expected if donor–acceptor interactions with residues of the protein stabilize the oxidized Fe^{IV} reaction intermediate. Evidence of additional haem-linked ionizations with p*K* values near 7.5 was also found, and this transition involves deprotonization of several groups of the protein, conversion of Fe from high to low spin, and possible denaturation of the protein.[381]

[378] R. Nagai, T. Kagimoto, A. Hayashi, F. Taketa, and T. Kitagawa, *Biochemistry*, 1983, **22**, 1305.

[379] D. L. Rousseau, M. R. Ondrias, S. L. Tan, T. Kitagawa, and E. R. Henry, *Raman Spectrosc. Proc. Int. Conf. 8th*, 1982, 725.

[380] H. Susi and M. D. Byler, *Biochem. Biophys. Res. Commun.*, 1983, **115**, 391.

[381] J. A. Shelnutt, J. D. Satterlee, and J. E. Erman, *J. Biol. Chem.*, 1983, **258**, 2168.

Resonance Raman data from deoxy-Hb and photodissociated HbCO (Hb*) at a temperature of 10 K using 457.9 nm excitation have shown that the Fe-histidine stretching mode in deoxy-Hb disappears, although with 441.6 nm excitation it is detected at 235 cm^{-1}. The intensity change apparently results from changes in energy of iron *d*-orbitals induced by the shortening of the Fe—His bond on temperature reduction. The previously reported narrowing of this Fe—His mode with reduction was found to be an artifact. In Hb* the Fe—His stretching mode is present with both excitation wavelengths and is at a higher frequency (242–244 cm^{-1}) than in the deoxy samples. Several porphyrin skeletal frequencies are also shifted in Hb* with respect to deoxy-Hb. These data apparently result from perturbations on the ligand-free histidine–porphyrin complex by the liganded conformation of the haem pocket. No evidence that the dissociated CO molecule interacts with the haem in Hb* was found.[382]

β-Lactoglobulin. The S—H, S—S, and C—S stretching frequencies of the sulphydryl groups of β-lactoglobulin have been measured using laser Raman spectroscopy. The S—H stretching band was observed at 2570 cm^{-1} and shifted to 1865 cm^{-1} in the presence of deuterium. The S—S stretching band of the two cystine linkages was observed at 537 cm^{-1}. The S—S and C—S stretching frequencies indicated that both cystine bridges have a *gauche,gauche,gauche* conformation.[383]

Lens Proteins. Age-related structural changes in the lens proteins of a normal mouse lens were monitored *in situ* by laser Raman spectroscopy. The Raman spectrum of an ICR-strain mouse-lens nucleus showed virtually no change in the 500–850 and 900–1800 cm^{-1} regions as the mouse aged. Lens ageing did, however, cause a significant intensity decrease of the Raman band at 880 cm^{-1} due to tryptophan residues, and the intensity decrease seems to be stepwise, implying that a microenvironmental change of these residues in the lens proteins takes place twice at different places during normal ageing. Particularly striking is that the intensity decrease of the band at 880 cm^{-1} proceeds in parallel with that of the Raman band at 2579 cm^{-1}, owing to a SH stretching mode. Thus the first microenvironmental change of tryptophan residues seems to be correlated with the formation of S—S bonds. In contrast to tryptophan residues, no evidence was observed for a microenvironmental change in tyrosine residues. In this respect, the structural changes of lens proteins in ageing are sharply distinct from those in lens opacification in which tyrosine as well as tryptophan residues undergo microenvironmental changes. The relative intensity of the band at 3390 cm^{-1} due to an OH stretching mode of lens water fell rapidly for the first four months and then decreased very gradually. The observation clearly exhibits the process of lens dehydration. The age-dependent profile of the relative intensity of the OH stretching mode is similar to that of the SH stretching mode, implying that lens dehydration is also related to the S—H to S—S conversion.[384]

[382] M. R. Ondrias, D. L. Rousseau, and S. R. Simon, *J. Biol. Chem.*, 1983, **258**, 5638.
[383] D. M. Byler, H. Susi, and H. M. Farrell, *Biopolymers*, 1983, **22**, 2507.
[384] Y. Ozaki, A. Mizuno, K. Itoh, M. Yoshiura, T. Iwamato, and K. Iriyama, *Biochemistry*, 1983, **22**, 6254.

Myoglobin. Multiple-internal-reflectance i.r. spectra have been recorded for variably hydrated films of the carbonyl and oxy forms of human haemoglobin and sperm-whale myoglobin. Even the limited removal of liquid and ice-like hydration constraints at the globin surface is sufficient to cause a dramatic but completely reversible shift towards a normally minute population of sterically unhindered, linear-perpendicular Fe—CO conformer modes [ν(CO) = 1968–1967 cm^{-1}] and the destabilization of distally hindered, tilted (or bent) Fe—CO modes [ν(CO) = 1951, 1944–1933 cm^{-1}]. Corroborative evidence from i.r. band-broadening trends, corresponding changes in the visible spectra, and H—D exchange kinetics confirm that the shift towards 1968–1967 cm^{-1} results in a more open distal-haem-pocket configuration and that it is also accompanied by a build-up of deoxy-like steric hindrance proximal to the haem. Denaturation effects are eliminated as a potential cause of the shifts, as are specific protein-protein, ion-protein, intersubunit, and multiple-internal-reflectance crystal-film surface interactions. The hydration effect exhibits globin- and ligand-dependent differences, which highlight the intrinsic importance of distal steric effects within the haem pocket and their dynamical coupling with exterior solvent constraint.

CO-photodissociation and O-exchange experiments conducted on rapidly interconverting and non-interconverting Fe—CO conformers also suggest that the open linear-perpendicular mode corresponds to a more tightly bound form of CO than the axially distorted Fe—CO species; similar differences are not evident in Fe—O, which already prefers a bent end-on geometry within the haem pocket. Control i.r. spectra aimed at monitoring the progressive effects of various denaturants on HbCO further indicate that this same mode serves as a common precursor to any of a number of more highly disordered folding modes. The overall properties of the 1968–1967 cm^{-1} conformer are discussed in terms of (1) the possibility of its corresponding to an available relaxation mode capable of facilitating the dynamics of ligand entry–release events and (2) its potential, added significance as a native folding mode that exhibits a marked tendency to be destabilized by hydration.[385]

The resonance Raman spectra of the hexa-co-ordinated sperm-whale Fe^{II}-nitrosylmyoglobin (I) were found to be independent of pH for both the 200–700 and the 1350–1650 cm^{-1} regions. In agreement with earlier e.s.r. results and contrary to recent resonance Raman results, these spectra did not indicate a transition to penta-co-ordinated I at low pH. However, interaction of I with SDS led to the formation of a pure penta-co-ordinated state with a typical resonance Raman spectrum. Replacement of ^{14}NO with ^{15}NO in penta-co-ordinated I did not induce isotope effects.[386]

Muscle. Raman spectra were obtained for an intact muscle fibre and for internally perfused fibres in capillary tubes. The use of internal perfusion ensured a good control of the concentrations of Ca^{2+}, Mg^{2+}, and ATP. The comparison of the spectra obtained with the two types of fibres shows that the

[385] J. W. Sutcliffe and P. D. Pulsinelli, *Biochemistry*, 1983, **22**, 2914.
[386] H. C. Hackin, B. Benko, J. T. Yu, and K. Gersonde, *FEBS Lett.*, 1983, **158**, 199.

muscle structure is well preserved. In addition it appears that the sarcomere length has no significant effect on the Raman spectrum of the fibres. Results on the perfused fibres demonstrate that a fibre can be kept in the relaxed state for several hours and then display an intact fibre spectrum when the concentrations of ATP, Mg^{2+}, and Ca^{2+} are maintained at 5 mM, 2 mM, and 0 mM, respectively. Therefore, ATP and Mg^{2+} do not affect the Raman spectrum of muscle fibres. When one of these components is removed or when Ca^{2+} is added, the contraction that occurs causes major spectral changes. These results are interpreted as being due to strong electrostatic interactions between toxic and acidic residues during contraction and to a change in the helical content or in the orientation of some of the contractile proteins.[387]

The analyses of the Raman spectra of purified myosin, C-protein, and myosin–C-protein complex in aqueous solution confirmed that C-protein adopts a predominantly non-α-helical structure whereas myosin adopts a predominantly α-helical one. Neither 10 mM $MgCl_2$ nor filament formation induced large conformational changes in purified myosin. Thermal denaturation of purified myosin, however, induced large changes in the Raman spectrum owing to conformational changes. Thus it is likely that the binding of myosin and C-protein is not accompanied by large conformational changes in the proteins.[388]

Protein Secondary Structure. – A new method for estimating protein secondary structure from the laser Raman spectrum was developed whereby the amide I Raman band of a protein is analysed directly as a linear combination of amide I bands of proteins whose secondary structures are known. For fourteen proteins, analysed by removing each one from the reference set and analysing its structure in terms of the remaining proteins, the average correlation coefficients between the Raman and *X*-ray diffraction estimates of helical, strand, turn, and undefined were 0.98, 0.98, 0.82, and 0.35, respectively. Significant correlations were also observed for distinctions between helix (0.98) and disordered helix (0.82) and between parallel (0.82) and antiparallel (0.97) sheets. The average standard deviation of the Raman estimates from the X-ray values is $<4\%$. In addition, a singular value analysis of twenty Raman amide I spectra indicates that there may be as many as nine significant independent pieces of information present in the amide I region.[389]

The use of second-derivative FT i.r. spectra to analyse the secondary structure of proteins has been referred to above.[380]

4 Circular Dichroism

Contributed by T. Brittain and A. Mathews

General. – *Reviews.* Few reviews of circular dichroism (c.d.) have appeared over the last year. Chiroptic methods in general have been reviewed,[390] together with

[387] J. P. Caille, M. Pigeon-Gosselin, and M. Pezolet, *Biochim. Biophys. Acta*, 1983, **758**, 121.
[388] K. Kishi and H. Noda, *J. Biochem. (Tokyo)*, 1983, **94**, 353.
[389] R. W. Williams, *J. Mol. Biol.*, 1983, **166**, 581.
[390] G. Snatzke, *Chem. Unserer Zeit*, 1982, **16**, 160.

the circular-dichroic activity of metal complexes.[391,392] Investigations of the structure and conformation of pigments bound to proteins as measured by c.d. have also been summarized.[393]

Instrumental. For the first time the use of c.d. as a means of detection in gas chromatography has been described.[394] Optico-acoustic detection of low levels of c.d. activity has also been discussed.[395]

Theory. In a series of papers Tinoco and his colleagues have investigated the effects of scattering of polarized light on the measured c.d. activity of large systems. It has been shown that the differential scattering of left and right circularly polarized light can make an important contribution to the measured c.d. of macromolecules, particularly if the dimensions of the particles are $>5\%$ of the wavelength of the light employed. Differential scattering effects are thus most important for viruses, membranes, and protein–nucleic acid complexes. Outside the absorption bands of the scattering, chiral particle only differential scattering contributes to the c.d. The sign and magnitude of the differential scattering are quantitatively related to the relative orientations and distances between the scattering units of the particle. The interpretation of these effects can be based on simple, classical methods. In fact, the measurement of the scattering, as a function of angle of circularly polarized light, can provide structural information on these systems by the application of general equations that relate this effect to the eigenfunctions of a scatterer of arbitrary size relative to the wavelength of light.[396,397] Experimental methods have been described to allow separation of the scattering component from the measured c.d. and its quantitation in the example of bacteriophage T2 in solution.[398]

A number of theoretical treatments of the c.d. of polypeptides and optically active amides have been reported. Using the Bogoliubov exciton theory a linear-response theoretical description of polypeptide c.d. has been derived. In this treatment the static field at each chromophore was approximated as an external field unaffected by the polarization it induces. Susceptibilities that contain the static-field effects were obtained *via* a Green operator method and used in the geometric expansion of time-dependent Hartree theory.[399] In a wider-ranging paper the present status of the theory of the optical properties of polypeptides was discussed. Arguments were presented to indicate that the interaction of

[391] U. Sakaguchi, *Gendai Kagaku*, 1982, **141**, 27.
[392] R. D. Peacock and B. Stewart, *Coord. Chem. Rev.*, 1982, **46**, 129.
[393] G. H. Wagniere, *Struct. Complexes Biopolym. Low Mol. Weight Mol., Proc. Workshop Conf. Hoechst, 11th*, 1981, 173.
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exciton bands in helical structures occurs in a tensorial fashion with the parallel polarized bands normally having stronger interactions than the perpendicular polarized bands, with only small cross-interactions. The net result is an anisotropy of the hyperchromicity of the π–π^* band and an imbalance of the rotatory strengths of the parallel and perpendicular components.[400] The simple intramolecular charge-transfer model for the electronic structure of amides has been extended to include the effects of torsion about the peptide bond ($\Delta\omega$) and pyramidalization at the peptide nitrogen (θ_N). The model has been used to calculate the c.d. spectra of non-planar amides, and the contribution of these to various polypeptide conformations has been discussed. The effects are predicted to be negligible for α-helixes but significant in the cases of β-sheet and β-turns. The theory could explain the c.d. spectra of unordered peptides if there were a bias towards negative $\Delta\omega$ values; however, evidence does not appear to support this required bias.[401] Empirical conformational-energy calculations performed on Ac-X^1-X^2-NHMe (X^1-X^2 = Pro-D-Ala, D-Ala-Pro, Pro-Ala, or Ala-Pro), Ac-X^1-D-Ala-Pro-X^2-NHMe ($X^1 = X^2$ = Ala, Gly, or D-Ala), and Ac-X^1-Pro-Ala-X^2-NHMe ($X^1 = X^2$ = same) when compared with c.d. data for the corresponding Dnp-Gly-X^1-X^2-Gly-pNA (Dnp = dinitrophenyl, pNA = $NHC_6H_4NO_2$-*p*, X^1-X^2 = same), Dnp-X^1-D-Ala-Pro-X^2-pNA (X^1 = Leu, X^2 = Val; $X^1 = X^2$ = Gly or D-Ala), and Dnp-X^1-Pro-Ala-X^2-pNA (X^1 = D-Ala, Gly, or Leu, X^2 = D-Ala, Gly, or Val) prove the validity of the chiroptic method using Dnp/pNA derivatives for studying β-turn tendency in peptide sequences.[402]

Green's function theory has been applied to the calculation of c.d. bandshapes. A modified Green's function method has been applied to the calculation of the aggregate bandshape whereby a decoupling approximation for vibrationally averaging the Green's function was used to include thermal and static effects as well, in terms of the correction shift of the chromophore bandshape. The utility of this method is exemplified by its application to the calculation of the c.d. bandshapes of a model dimer system.[403] A Green's function formalism has also been used to predict the contribution of the intrinsic c.d. to the total c.d. of an aggregate of chromophores. The intrinsic c.d. was determined from the monomeric c.d. and corrected when comparing for effects of the intermolecular interactions in the aggregate using a first-order correction to the Born–Oppenheimer approximation.[404] The general lineshapes for both natural and magnetic c.d. have been expressed in a resolvent formalism with specific expressions presented for the resulting lineshapes for a Fano-type interference model, where a discrete excitation is coupled vibronically to an overlapping highly dense or continuous excitation.[405]

A recently developed statistical-mechanical theory for the treatment of the α-helix to random-coil transition in two-chain α-helical proteins has been applied to extant c.d. data on the thermal transition in both crosslinked and non-

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crosslinked α-tropomyosin, together with thermal data on the unfolding of two tryptic fragments T1 and T2. For the case of the whole protein this theory has proved to be effective in the separation of effects due to mass action and local distortions when comparing the non-crosslinked and crosslinked proteins.[406,407]

An extended dimer model has been formulated that allows for mixing of monomer electronic states by intermolecular interaction. When applied to dimers, the model readily predicts the observable changes in vibronic structure produced by the transfer of intensity and rotary power due to inter-state mixing.[408]

The rotatory strength of the $n \rightarrow \pi^*$ transition in chiral molecules containing a carbonyl group has been computed using the CNDO/S technique, and electrostatic solvent effects on this transition have been quantified.[409]

Semi-empirical MO calculations have been made to obtain information about the spectroscopic properties of Congo Red and Cibacron Blue. Calculations of rotational strengths for chiral conformations, selected to mimic the conformation of NADH bound to dehydrogenases, yielded values compatible with the observed c.d. of the enzyme-bound dyes, indicating that the intrinsic contributions could be responsible for the observed c.d. Moreover, calculations on the coupling of the dye transitions with the π–π^* transitions of an indole group showed that coupling with a protein is not likely to be a significant factor in the induced c.d. of Congo Red but may be comparable in size to the intrinsic contributions for Cibacron Blue. The implications of these results on the interpretation of dye–dehydrogenase-binding studies were discussed.[410]

Small Molecules, Model Compounds, and Synthetic Polymers. – *Amino Acids and Derivatives.* A rapid method for the determination of absolute configuration and quantity of amino acid enantiomers in mixtures has been presented. The absolute configuration is derived from the sign of the c.d. at 340 nm following reaction of the amino acid mixture with *O*-phthalaldehyde in the presence of mercaptoethanol. This method is simple and allows unambiguous determinations on samples down to 2×10^{-5} M.[411]

Ac-X-NHMe (X = Phe, Tyr, or Trp) has been used as a model compound for aromatic amino acids in proteins. An increase in temperature or decrease in solvent polarity was found to give similar effects on the amide $\pi^* \leftarrow n$ transition c.d. When X = Phe or Trp, the temperature-dependent c.d. in the 240–300 nm region has been interpreted in terms of the coexistence of various conformers at high temperatures.[412]

Once again this year, transition-metal complexes of amino acids have proven to be an area of significant research activity. A series of papers has appeared

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concerned with the properties of cobalt(III) complexes of amino acids. Four glycinato, 6-L-alaninato, and (*R*)-propylenediamine cobalt(III) complexes of a tripod-like quadridentate ligand *N,N*-bis-(2-aminoethyl) glycinate have been prepared, and the absolute configurations of the chiral complexes have been assigned from the measured c.d. data.[413]

In a closely related investigation the geometrical configurations of an analogous series of complexes using the tripod-like *N*-(2-aminoethyl)iminodiacetate ligands have been established.[414] Solvent effects on the stereoselectivity between Λ- and Δ-β_2-diastereoisomers of Co^{III} complexes with the general formula [CoL(L-aa)] [H_2L = (o-$HOC_6H_4CR{=}NCH_2)_2$, R = H, Me, or bis-(naphthylidene)ethylenediamine, L-aaH = L-Ala, L-Val, L-Met, or L-Phe] have been reported.[415] The c.d. behaviour of the complexes $[CoL_2]^+$ [HL = *S*-methyl-D-penicillamide (D-smpH) or *S*-methyl-L-Cys (L-smeH)] and $[Co(D\text{-}pen)_2]^-$ (D-penH_2 = pencillamine) has been discussed in relation to the comparison of thioether and thiolato complexes.[416]

Studies on the sulphur complexes of Pd^{II} employing L-Met, *S*-Me, *S*-Et, and *S*-benzyl-L-Cys as ligands have shown that the nature of the sulphur-atom substituent has a critical role to play in determining the absolute configuration of the sulphur atoms in the complexes.[417,418]

The presence of substantial differences in the optical activity of PtL (HL = Ala, Ile, D-Leu, NorVal, Pro, or Val) and PtProl (HL = Ala, Sar, Ser, or Val) between their aqueous solution and corresponding solid polycrystalline states has been interpreted as indicating the presence of strong intermolecular interactions in the solid samples.[419]

C.d. studies have allowed the assignment of the co-ordination structures of various species in the Cu^{II}–L-His (1 : 2) system as a function of pH. Using ternary complexes of Cu^{II}–histamine-L-His and Cu^{II}–amino acid-L-His, it has been possible to identify the characteristics of the possible glycine- and histamine-like binding modes of L-His. This information shows that $Cu(HL)^{2+}$ (HL = L-His), the major species below pH 3, contains a glycine-like bound His. CuL_2, the major species at neutral pH, exists as an equilibrium mixture of a mixed-type chelation structure. The imidazole-containing species present at pH ~11 shows an increasing glycine-like contribution.[420] The Cu^{II}–L-*N*-MeHis series shows co-ordination characteristics similar to those of L-His, whilst the Cu^{II}–L-N^{α},N^{α}-diMeHis series shows significant differences. The anionic form of the diMeHis

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ligand is bound glycine-like, and the species present at neutral pH also reflects this ligand's higher preference to bind in a glycine-like fashion.[421]

The coupling of the asymmetry of the L-His groups to the Mg porphyrin in the bis-histidinate complex produces large induced Cotton effects, whilst the five-co-ordinate systems show no such effects. These results lend support for the coupled oscillator description of the origin of optical activity in haemoproteins.[422]

The c.d. of the Schiff-base complexes of pyridoxal with fifteen L-amino acids in MeOH showed that the predominant conformation of the C_α—N bond is approximately the same for all the compounds and involves a pseudo-equatorial disposition of the C_α—H bond with respect to the plane of the extended π-system. The sign of the Cotton effect near 425 nm varies with the nature of the side chain, according to the chirality of the dominant interaction of the amino acid residue with the *N*-pyridoxylidene chromophore.[423]

Dipeptides and Oligopeptides. C.d. spectra at sub-zero temperatures of Ac-Trp-Trp-NHMe, Ac-Phe-Phe-NHMe, and Ac-Tyr-Tyr-NHMe indicate a folded structure in 2,2,2-trifluoroethanol but an extended structure in tetrahydrofuran. These results were used for comparison with calculations predicting the low-energy conformations for these systems.[424]

Cyclo(Ala-Gly), cyclo(Ala-Ala), and cyclo(Pro-Pro) show a number of intense c.d. bonds in the vacuum u.v. region below 180 nm in addition to the usual amide c.d. bonds. Independent calculations showed that these short-wavelength bands can be attributed to the σ–σ^* transitions of the backbone but cannot be accounted for by short-wavelength transitions of the amide bonds.[425]

The effects of substituents in $[Co(NH_3)_2$(L-Ala-Gly-Gly)$]\cdot 2H_2O$ and analogous complexes on the measured c.d. spectra have been discussed in terms of different contributions to the chirality, including side-group rotameter populations and the non-planarity of the chelate ring systems.[426]

From c.d. measurements of the S—S bond transition it appears that the observed red shift seen on cation binding to the bis-cyclic tetrapeptide *S,S'*-bis[cyclo(Gly-L-hemi-Cys-Sar-L-Pro)] indicates a change in peptide backbone geometry and covalent CSSC bridging upon complexation with the metal cation.[427]

Titration curves followed by c.d. spectroscopy showed that cyclo{Gly-L-Cys[Bzl(OMe)]-Sar-L-Pro} [Sar = MeGly, Bzl(OMe) = $CH_2C_6H_4OMe$-*p*] in MeCN forms at least three different types of complex with Ca^{2+} and Ba^{2+} but only a 1:1 complex in the presence of Li^+ and Mg^+. In all cases the conformational change observed on metal binding involves changing the geometry of the peptide bonds around the Cys-Sar-Pro sequence.[428]

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Side-chain contributions to the far-u.v. c.d. of Asn-D-Ala-Gly-Ala-NH_2, Asn-Ala-Gly-Ala-Asn, and a variety of peptides have been described.[429]

In Boc-$(X)_n$-OEt (Boc = Me_3CO_2C; X = Ala or Leu, n = 2–8; X = Val, n = 2–7) β-structures are observed at $n = 8$ for the X = Ala compound and $n = 7$ for the X = Val compound in trifluoroethanol. In the case of the Leu series β-structures did not appear even for $n = 8$. Increasing temperature or dilution was found to decrease the stability of these β-structures.[430]

Oligopeptide fragments of glucagon, substance P, and α-MSH have been studied with regard to the effect of terminal residues on the tendency of peptides to form ordered structures in sodium dodecyl sulphate (SDS). In 25 mM SDS approximately nine amino acids are necessary to produce a helical structure, whereas only eight are needed in 1 mM SDS.[431] The role of peptide chain length and specific amino acid sequence in determining peptide solution conformation has also been studied in emcrimicin antibiotic fragments (2–9) and in $(Aib)_3$-L-Val-Gly-L-Leu-$(Aib)_2$, benzyloxycarbonyl-$(Aib)_3$-L-Val-OMe, and benzyloxycarbonyl-$(Aib)_3$-L-Val-Gly-OMe (Aib = aminoisobutyric acid). C.d. data show that the tri-pentapeptides adopt a 3_{10} helical structure in solution whereas the octapeptides adopt an α-helical structure.[432]

Solvent-induced differential binding of metal cations has been demonstrated in Me_3CO_2C-Cys(Acm)-D-Leu-His-Cys(Acm)-D-Leu-His-OMe and its cyclized counterpart. In water the c.d. of both forms of the peptide is altered by the addition of Zn^{2+} but not of Ca^{2+} or Mg^{2+}. However, in methanol solution Zn^{2+} and Ca^{2+} were effective but Mg^{2+} was not.[433] The trianionic forms of a series of cobalt complexes of the form $NH_4[Co(tetrapeptidato)(NH_3)_2]$ containing L-Ala and/or Gly show additivity of their vicinal c.d. spectra.[434]

The 2-nitrophenylsulphenyl chromophoric derivatives of α-amino acids have been shown to be good probes for the β-structure in oligotyrosine peptides using Nps-$[Tyr(Bzl)]_n$-Gly-$OCMe_2CH_2$OPEG-M [Nps = 2-nitrophenylsulphenyl, Bzl = CH_2Ph, OPEG-M = poly(ethylene glycol) monomethyl ether, n = 3–8] as a model system.[435]

A range of tyrosine peptides and derivatives have been used in the study of the optical properties of the ^1L_b band of the phenolic group. C.d. recorded at different pH values in the 240–320 nm range has been discussed in terms of charge effects arising from the N- and C-terminal groups, together with local conformational influences on the ^1L_b band. The possible existence of a correlation between observed ellipticity and rotamer populations in these samples has been examined.[436]

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In acid aqueous solution H-Asn-Ala-Gly-Ala-Asn-OH shows a positive c.d. peak at 215 nm and a negative peak at 232.5 nm assigned to a β-structure. In 90% ethanol or trifluoroethanol at pH 5.8 this compound shows a negative peak at 218 nm, the intensity of which is independent of concentration. These phenomena have been interpreted as arising from the formation of an intramolecular β-pleated sheet-like structure. H-Asn-Ala-Gly-Ala-OH shows a single positive band at 215 nm; H-Asn-D-Ala-Gly-Ala-NH_2 exhibits only a negative band at 196 nm, assigned to β-turn and random-coil conformations, respectively.[437]

Icosa-L-Lys and poly-L-Lys conjugates with benzylpenicillin in H_2O and CF_3CH_2OH exhibit considerable right-handed α-helical content. Difficulties encountered in obtaining fully penicilloylated conjugates do not, however, appear to be related to the extent of α-helical content.[438] It has been shown that interchain β-structure occurs at d.p. 4 in homo-oligopeptides of ε-(benzoyloxycarbonyl)-(L-Lys)$_n$ in water and that right-handed α-helical structure occurs at d.p. 6 in CF_3CH_2OH, but this can be converted to the β-structure by the addition of water to the CF_3CH_2OH solution.[439]

The collagen-like polytripeptide (Pro-OH-Pro-Gly)$_{10}$ exhibits a relatively small negative rotation at 578 nm but does not show a thermal transition as is seen with collagen or other collagen-like polytripeptides that form triple helices. Triple-helical character was evident, however, at low temperature as seen by the positive peak at 224 nm and negative peak at 200 nm. The intensity of these features was significantly less than those expected for full triple-helix formation. Comparison of this compound with other polytripeptides demonstrates that Pro-OH in the X-position decreases the thermal stability of the triple helix. This finding cannot be explained by current models for the structure of the triple helix.[440]

Using the host–guest technique, tentative scales for the helix-inducing power and β-structure-forming potential of various side-chain-protected amino acid residues have been established.[441]

Polypeptides. The induced c.d. of poly(L-Lys)–azo dye complexes becomes less negative as the length of the aliphatic chains on the dye increases. *N*-Aliphatic anilines or naphthalene derivatives of azo dyes are necessary to induce c.d. in the complexes, and small substituents on the dye cause large effects.[442] Poly(ε-*p*-toluenesulphonyl-L-Lys) takes up a right-handed α-helix in solution. Thin solid films exhibit extrinsic c.d. that is induced by a chiral array of the side chains.[443]

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A range of branched polypeptides based on a poly(L-Lys) backbone assume an unordered conformation at neutral pH and low ionic strength. An increase in ionic strength to 2M leads to the formation of the α-helical structure. α-Helix formation is supported by the presence of L-Leu and L-Pro but is limited to some extent by the presence of His, particularly at lower ionic strengths.[444] 'Statistical' copolymers of L-Leu and L-Lys with 50 mol % Leu form α-helices at low ionic strengths (0.003M $LiClO_4$ or $NaClO_4$) but not in pure water. In contrast with basic α-amino acid homopolymers, sulphate in this case also induces α-helical structure. The effect of sulphate is rationalized in terms of its known effects on water structure.[445] The analogous alternating Leu-Lys copolymer shows a coil-to-β transition induced by high pH or the presence of various salts. In the presence of ClO_4^- an intramolecular cross-β-structure is formed, whereas SO_4^{2-} produces an intermolecular β-pleated-sheet structure. Both of these β-structures are stable up to 90 °C.[446] In trifluoroethanol and alcohol–water mixtures alternating copolymers of (L-Leu-L-Lys) and (L-Leu-L-Orn) both reveal a tendency to form α-helices. The α-helix-inducing power of various alcohols was found to be in the order trifluoroethanol > PriOH > EtOH > MeOH. In MeOH–water mixtures high pH favours β-structures at <50% MeOH and α-helix at >60% MeOH.[447]

Poly(α-L-Glu) undergoes two conformational transitions in the presence of Cu^{II} in the pH range 4–7; the helix–coil transition and aggregate formation associated with completion of the helix. The pH dependence of the molar ellipticity has been analysed in terms of the two-state model, which showed that this description is inadequate to describe the helix–coil transition.[448]

Na poly(L-Glu)–Cd^{2+} is present in solution as an α-helix at low pH but is converted to the β-form at high pH. The residue ellipticity at 230 nm, at a Cd-to-peptide ratio of 10, decreases slightly with increasing temperature, but the c.d. spectrum remains essentially the same as that of the β-form even at 80 °C.[449]

Random copolypeptides composed of (hydroxyethyl)-L-glutaminyl residues show c.d. spectra expected for partially helical polypeptides in water over the range 4–64 °C. The helicities observed experimentally correlate well with those predicted from theoretical considerations.[450]

Poly(γ-ethyl *N*-methyl-L-Glu) shows a negative band at 229 nm and positive bands at 200 and 175 nm, which suggests that the polypeptide assumes the three-fold right-handed helix and rules out the possible poly(L-ProIII) conformation.[451]

C.d. titrations show that poly(α,β-L-Asp) undergoes a complex pH titration with the α-helix form stabilized at low degrees of ionization. The extent of the conformational changes associated with the titration depends on the mole

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fraction of β-bonds present, with a high mole fraction favouring a lower helix-forming ability.[452]

Proline-rich polypeptides containing more than four consecutive Pro residues, obtained from human saliva, take up the poly-L-Pro form II conformation, as demonstrated by their c.d. spectra.[453]

Proteins. — *Non-chromophoric Proteins.* α_2-Antiplasmin appears to cause similar changes in the conformations of plasminogen and plasmin as does 6-amino-hexanoic acid, suggesting that a lysine-binding site is probably involved in such interactions. The disulphide bonds in native α_2-antiplasmin appear to have a minor role in controlling the native conformation.[454] The c.d. spectra of complexes of α_2-antiplasmin with aprotinin in the aromatic region are the sum of the contributions of each component, thus showing the absence of any hydrophobic interactions. C.d. in the backbone region, however, shows that major conformational changes occur on complex formation.[455]

Perturbation of both buried and exposed tryptophan residues occurs on binding of oligosaccharides to antithrombin. The use of oligosaccharides of various sizes has allowed the identification of two binding domains, involving S—S bonds or tryptophan residues, which are responsible for rapid binding with thrombin or the activation of antithrombin.[456]

Structural studies on human urinary urokinase in relation to changes in plasminogen-activator activity show that the denaturation of the enzyme occurs in three transitions. Addition of 0.75–1.25M guanidinium hydrochloride to the native enzyme leads to a transition to an activated form with an 18% higher urokinase activity.[457]

Near-u.v. c.d. spectra have provided important information about the environments of the aromatic and disulphide side chains in the refolding processes of bovine pancreatic trypsin inhibitor. Although secondary structures of the refolding intermediates could not be unambiguously assigned, all the spectra showed that non-random conformations gradually appeared as disulphide-bond formation progressed.[458]

The maintenance of the correct disulphide-bonding pattern in Kunitz soybean trypsin inhibitor has been shown to be essential to the protein's trypsin-inactivating activity.[459]

α_2-Macroglobulin shows a 5% decrease in α-helical content, as compared with the native molecule, when treated with either primary amines or proteinases. It is suggested that inactivation by the primary amines and complex formation both occur *via* the same spatial rearrangement of the α_2-macroglobulin sub-

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units.[460] In a very similar study using difference c.d. spectroscopy in the u.v. region it was shown that the binding of thrombin to α_2-macroglobulin perturbs ~5.4 tryptophan residues, whereas reaction with methylamine does not lead to these alterations. Kinetic investigations also show the close similarity in the alterations in conformation induced by proteins and methylamine.[461] It has been found that the addition of Na^+ to solutions of human α-thrombin leads to an increase in the ordered protein structure whereas Li^+ has the reverse effect. This behaviour is consistent with the effects of these cations on the autolysis rate and thermal stability of α-thrombin.[462]

The α-globulin from *Sesamum indicum* has ~25% β-structure and 5% α-helix. Detergents such as CTAB and SDS both increase the α-helical content of the protein, whereas non-ionic detergents such as Brij-36T and Triton X-100 form specific β-structures such as II-β and I-β within the protein. The protein apparently has a fairly labile quaternary structure.[463]

The binding constant of Ca^{2+} of bovine, human, and goat α-lactalbumins in the native-like apo form has been determined and compared with similar binding parameters determined from renaturation studies on the thermally denatured protein.[464]

A range of studies have been carried out on structural proteins. The c.d. of bovine-plasma fibronectin in the 200–250 nm range has been assigned solely to tyrosine side chains. Some of these tyrosine residues appear to be located in structured domains that undergo conformational changes on binding to heparin. These studies may prove useful in investigations of the interactions of fibronectins and related glycoproteins of the extracellular matrix.[465] Human fibronectin adopts a different conformation when bound to phospholipid vesicles from when in aqueous solution, owing to specific interactions and not merely hydrophobic effects. These studies suggest that the physiological role of this protein may be promoted by lipid molecules and may not require the action of an intermediate receptor.[466] Vincristine binds stoicheiometrically with calf-brain tubulin, inhibiting its self-assembly into microtubules without significantly altering the protein's conformation.[467] Near- and far-u.v. c.d. shows that, although tubulin can form conformations with high helical content in apolar conditions, this transformation is not involved in self-assembly. The absence of any major perturbations in the near-u.v. c.d. under conditions of assembly has been interpreted as being consistent with a model involving direct incorporation of oligomers rather than the alternative condensation polymerization scheme.[468] A direct correlation between crossbridge release and the α-helix–coil transition

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has been established in myosin.[469] The three bovine-neurofilament subunits show specific c.d. activity reminiscent of that obtained for α/β-proteins. Furthermore the good correlation among c.d. spectra, state of renaturation, and the ability of proteins to assemble into filamentous structures should prove very valuable in unravelling the effects of the numerous variables affecting the disassembly and reassembly process.[470]

The binding constants of a range of both anionic and non-anionic substrates to baker's yeast transketolase have been determined. A comparison of K_m and V_{max} values for the various substrates shows that the donor-substrate anionic group, which is essential for binding, does not affect the catalytic rate.[471] Modification of the enzyme with butane-2,3-dione does not alter the K_m values obtained but decreases V_{max} values by 5–10-fold. Apparently an arginine residue is essential for catalytic activity but plays no significant role in the substrate-binding process.[472,473]

Bovine-brain aldolase C binds methylmercuric acetate at four sulphydryl sites. Below this level of binding the presence of the mercury salt induces a partial β-random-coil transition. At mercury concentrations greater than those necessary to bind four SH groups the protein undergoes a significant structural change with the α-helical content being reduced from 30% to 9.5%, with a concomitant increase in β-structure to 50% and in random-coil structure to 40.5%.[474] The monomeric class I fructose 1,6-bisphosphate aldolase from *Staphylococcus aureus* unfolds at relatively low temperatures (26–39 °C) and also in the presence of 6M guanidine hydrochloride. Reactivation of the enzyme occurs in <10 s at 25–27 °C, which rules out the possibility that isomerization around at least some of the ten X-Pro peptide bonds present in the enzyme is rate-limiting in this process.[475]

Both human-plasma lipoprotein A and β_2-glycoprotein I show protein structures that differ significantly from those determined for other well characterized apolipoproteins such as low- and high-density lipoproteins.[476,477] The secondary structure of human-serum apolipoprotein β is very similar to that of holo-low-density lipoprotein in the presence of phosphatidylcholine. Additions of cholesterol to this binary mixture lead to significant alterations in the c.d. spectra, presumably due to the incorporation of an uncharged part of the protein into a hydrophobic core.[478]

Although bovine-brain S-100a and S-100b proteins both undergo conformational changes upon binding of Ca^{2+}, the two proteins show distinct c.d.

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spectra in both situations. These differences have been assigned to the presence of a single tryptophan residue in the S-100a protein,[479] although differences in the environments of tyrosine and phenylalanine residues have also been revealed by near-u.v. c.d.[480]

At above pH 9.0 salting-in of tyrosine residues in RNase A and S enhances the contribution of these residues to the c.d. spectra of these proteins, leading to an apparent titration curve that is shifted towards lower pH. An increase in ellipticity at 275 nm on partial unfolding in salts was observed at pH 7 → 4. This observation may suggest that the isothermal unfolding at low pH proceeds *via* an intermediate step that involves histidine residues and causes conformational changes in the proteins' tyrosine environment.[481]

Factor H exhibits an unusual c.d. spectrum with a positive peak at 230 nm and a negative peak at 190 nm with no apparent α- or β-structure present. However, when the disulphide bonds were reduced followed by either reoxidation or alkylation the protein exhibits conventional c.d. characteristics. Disulphide-bond rupture removes the protein's regulatory functions. Minor conformational changes occur when Factor H binds with complement component C2b in solution.[482]

85% of the amino acids in the *lac* carrier protein from *E. coli* are arranged in helical secondary structures. These data, together with molecular size, suggest that the protein may exist as twelve α-helical segments that all traverse the membrane in a similar fashion to that seen for bacteriorhodopsin.[483]

Titrations for 25 protein–dodecyl sulphate complexes, over the region of COOH group ionization, show a significant increase in α-helix content on acidification. For the majority of proteins studied the spectral changes agree with the expected helix propagation by unionized glutamyl and aspartyl residues. In the cases of gastrin, Kunitz trypsin inhibitor, and tropomyosin, however, changes in charge density appear to be the controlling factor in protein conformation.[484]

Native phospholipase C and apophospholipase C from *Bacillus cereus* undergo unfolding at concentrations of guanidinium chloride of 1.4–1.8M and 0.1–0.6M, respectively. Apoprotein retains its near-u.v. c.d. spectrum even after all ordered structures have been destroyed. The apoprotein also binds two equivalent Ni^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Pb^{2+}, or Cu^{2+}, but only the latter causes any significant alterations to the observed c.d.[485]

Pig-intestinal calcium-binding protein exhibits some anomalous ionization behaviour. The protein possesses a single surface tyrosine residue, which c.d. investigations show does not ionize below pH 12.[486]

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Bacteriophage λ gene *cro* protein contains ~35% α-helix, 20% antiparallel β-structure, and 15% β-turn in the pH range 4.5–10.5. At more extreme pH or in the presence of 0.6–1.9M guanidinium chloride the protein shows an abrupt structural change of symbatic character. A possible mechanism of denaturation has been proposed.[487]

The structural content and thermal and salt stability of bovine-liver ornithine transcarbamylase show that the protein possesses considerable conformational stability. Inactivation of the enzymic activity of the protein appears to precede the unfolding process.[488]

The protein toxin from the sea anemone *Radianthus macrodactylus* is so stable that ionization at pH 12 or heating to 80 °C does not alter its conformation sufficiently to obviate its toxicity.[489]

Thrombospondin contains at least twelve binding sites for Ca^{2+}, which interact co-operatively (Hill coefficient = 12.3) in such a manner as to produce a small change in secondary structure subsequent to binding.[490]

Beef-liver arginase shows noteworthy stability. Although removal of Mn^{2+} or acidification below pH 4 completely destroys the enzymic activity of the protein, only limited alterations occur in the far-u.v. c.d. at pH 1.5. As determined from the temperature dependence of the c.d. signal at 220 nm the protein has a temperature of half inactivation of 77 °C in the presence of Mn^{2+} and 55 °C in its absence.[491]

The c.d. spectrum of modeccin from *Modecca digitata* is similar to the ricin and abrin toxins, with near-u.v. bands, assigned to buried tryptophan, being very little affected by high pH. The protein structure is converted to a higher % α-helix by the addition of SDS or trifluoroethanol.[492]

A comparison of c.d. measurements with theoretical predictions for the structure of the P_2 protein of peripheral nervous-system myelin has led to a general model of a Greek key β-barrel, consisting of eight antiparallel β-strands with a two-stranded ribbon of antiparallel β-structure emerging from one end.[493]

On cleavage from native thermolysin, fragment 121–316 retains its native helical content and presents remarkable thermal stability with a T_m of 74 °C, as assessed from the temperature dependence of the c.d. signal at 220 nm.[494]

Owing to a lower proline content α_{S2}-casein exhibits a higher α-helix content than does α_{S1}-casein.[495]

The fact that glutaric, succinic, or valeric acids protect the glutamate decarboxylase of *E. coli* from modification with butane-2,3-dione and concomi-

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tantly preserve the c.d. activity at 420 nm has been interpreted in terms of the presence of an arginine residue at the active site of the enzyme that interacts with the distal carboxylic group of the substrates.[496]

Although c.d. progress curves for the oxidative renaturation of reduced lysozyme appear independent of the nature of the oxidizing system employed, other kinetic detection systems indicate differences. Apparently these results arise from differences in the relative amounts and/or kind of reaction intermediates present in each case and lead to different mechanisms of renaturation that depend on the nature of the oxidant present.[497]

Native $C\bar{1}$-esterase inhibitor possesses a relatively high level of ordered structure that is reduced by gradual cleavage of the disulphide bonds under non-denaturing conditions. Complex formation between $C\bar{1}$ s and $C\bar{1}$-esterase inhibitor appears to alter the local environment around tryptophan residues within the protein.[498]

It is possible to obtain some information on the tertiary structure of proteins using c.d. spectroscopy. It has been shown that c.d. can distinguish between four classes of protein (predominantly α-helical, predominantly β-sheet, separate α- and β-rich regions, and intermixed segments of α- and β-structure) as each class has certain common features in its u.v. c.d. spectra.[499]

Chromophoric Proteins. A c.d. band has been identified in the spectrum of several fish haemoglobins at 416 nm. A pH-dependent change in the intensity of this feature occurs at around pH 6.0, and as such is not directly related to the known $R \rightarrow T$ transition that occurs at ~1 pH unit later.[500] pH titrations on trout IV haemoglobin, in the CO-bound form, have been associated with the previously identified $R_4 \rightarrow T_4$ transition. C.d. at >500 nm, at low pH, has been interpreted as being due to the release of haem asymmetry in the T-liganded state.[501] The assembly of subunits to produce tetrameric haemoglobin has been studied by c.d. in a number of derivatives. Assembly of oxidized α- and β-subunits to give the symmetric dimers $\alpha_1\beta_1$ and $\alpha_2\beta_2$ produces significant alteration in the Soret-region c.d. Further association to give tetrameric species produces very little further change.[502] The assembly process associated with the production of both oxy- and deoxy-haemoglobin from the constituent subunits has been followed using stopped-flow c.d. measurements. In the case of the formation of deoxyhaemoglobin, c.d. measurements identified a fast process in the Soret region ($k = 6.3 \times 10^5 M^{-1} s^{-1}$) that was rate limiting and probably arose from the combination of α- and β-subunits into dimers. A slow process apparent in the u.v. region ($k = 1 \times 10^4 M^{-1} s^{-1}$) was assigned to the tetramer production from two dimers.[503]

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In the case of the association of oxy subunits, aggregates of β-chains appear to be kinetically significant. A fast process associated with α,β-combination ($k = 7.5 \times 10^5 M^{-1} s^{-1}$) is observed together with a slow monomolecular process ($k = 2 \times 10^{-3} M^{-1} s^{-1}$) assigned to dissociation of aggregated β-chains.[504]

Formaldehyde has been shown to produce a reversible denaturation of myoglobin in which the helix–coil transition, unlike the case of denaturation by pH, urea, temperature, *etc.*, is non-co-operative.[505] The addition of Co^{2+} and Fe^{2+} phthalocyanines to apomyoglobin yields a protein with a secondary structure very similar to that of the native protein. Addition of the Cu^{2+} derivative, on the other hand, yields a protein with an increase in unordered structure that may be derived from the deformation of the phthalocyanine structure by the presence of Cu^{2+}.[506]

C.d. studies, together with n.m.r. measurements, have allowed the identification of an *S*-chiral methionine ligand in the cytochromes c_{551} from *Pseudomonas mendocina* and *Pseudomonas stutzeri*, whereas the methione ligand in cytochrome c_2 from *Rhodospirillum rubrum* is present in the R-chiral form.[507]

Based on model studies, it appears that the inactivation of cytochrome P450, by alkylation of the haem group, occurs on ring C for reagents containing more than six carbon atoms.[508]

The all-or-none denaturation and renaturation of porcine erythrocyte catalase correlate well with changes in the Soret c.d. bands. Near-u.v. c.d. shows that the recovery of α-helical structure precedes the ordering of the haem site and associated enzymic activity.[509]

A comparison of absorption and c.d. data shows that the activity of spinach ferredoxin requires a native protein structure that is maintained through interaction with the Fe—S cluster and protein structure in the neighbourhood of the cluster; both are independent of ionization of surface residues. An alkaline transition ($pK = 10$) in spinach ferredoxin produces changes in the Fe—S cluster microenvironment but does not alter the protein's overall secondary structure.[510] A catalytic role has been suggested for the micellar phase in the core extrusion from spinach ferredoxin. Kinetic and equilibrium measurements have been made in the presence of dimethyl formamide/Triton X 100 mixtures.[511] Fragmentation studies on thioredoxin showed that at low pH the 1–37 fragment exhibits the c.d. characteristics associated with the native protein. The possible mechanism for refolding of the short fragments was discussed.[512]

Spectral analysis of purified, isoelectric, forms of bovine rhodopsin in the 190–800 nm region indicates the presence of different tertiary structures in the

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different forms but essentially identical secondary structures.[513] Detergent treatment of bacteriorhodopsin removes the negative exciton c.d. band at 600 nm, but this effect can be inhibited by the presence of glutaraldehyde.[514] Further spectral studies on bacteriorhodopsin secondary structure suggest a modification of the seven-helix model for the protein to one in which five α-helical segments are connected by four strands of β-sheet structure.[515] Nitration of tyrosine residues in bacteriorhodopsin appears to alter the interactions of neighbouring chromophores normally present in the protein.[516]

Kinetic c.d. studies on the binding of Cu^{2+} to apoazurin indicate the presence of at least two intermediates in the binding process. At low pH the process arising from histidine ligation of Cu^{2+} is dominant; at high pH, however, cysteine ligation is dominant. The relevance of these results to the question of the selective incorporation of Cu^{2+} into the protein has been discussed.[517] The c.d. spectrum of native Cu–Zn superoxide dismutase has been reassigned on the basis of recent studies of the electronic spectra of model systems.[518] On the basis of studies of mercurial-binding to *Neurospora crassa* copper metallothionein and its effects on the protein's chiroptic properties it appears that the Cu^{I} ions co-ordinate to cysteinyl residues in the form of a single metal cluster.[519]

Using Ni^{II} as a probe of the bonding domains between the catalytic and regulatory subunits of aspartate transcarbamylase it has been possible to follow at 360 nm the binding to the six active sites of the bisubstrate *N*-(phosphonacetyl)-L-Asp. Features at 406 and 460 nm, in contrast to those at 360 nm, appear to reflect gross conformational changes due to ligand binding, which are complete at only 60% occupancy by the ligand.[520]

A good correlation exists between the FAD content of NADH–cytochrome b_5 reductase, c.d. activity at 460 or 285 nm, and enzymic activity. Reduction of the enzyme with NADH causes a reversal of the sign of the c.d. signals in the 300–500 nm region owing to the formation of a reduced FAD–NAD charge-transfer complex. SH-group-specific reagents identify the involvement of cysteine in the maintenance of the native enzyme structure and probably in the binding of NADH.[521]

Major differences occur between the c.d. spectra of the ternary complexes of lactate dehydrogenase, NAD, and pyruvate or α-ketobutyrate and those of the corresponding binary complexes, which disappear on dissociation of the ternary complexes with urea. This behaviour has been explained by proposing the formation of a NAD-activated transient in the abortive ternary complex.[522]

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The formation of the holo-pyruvate dehydrogenase complex with thiamine pyrophosphate is accompanied by the appearance of a new c.d. band at 320–360 nm, which has the characteristics of a charge-transfer transition between a peptide tryptophan residue and the thiazolium ring of thiamine. Thiamine pyrophosphate binds at two active sites with different affinities.[523]

C.d. has been used to follow the light-induced rearrangement of leaf membrane into grana and the associated accumulation of light-harvesting chlorophyll *a*/*b*-protein complex by monitoring the increase in intensity of the positive band at 682–684 nm and the negative band at 665–672 nm.[524] The symmetric positive and negative bands centred at 669 nm in the water-soluble chlorophyll-protein complex (CP663S) arise from the interactions between chlorophyll *a* molecules over short distances.[525]

Hormones. – Above 250 nm the c.d. spectra of all nine homologous and heterologous $\alpha\beta$-recombinants obtained from ovine and porcine lutropin and human choriogonadotropin more closely resemble those of the β-subunit than of the α-subunit. Difference spectra above 250 nm derived by subtracting the sum of the subunit spectra from the recombinant spectra all show a negative band near 280 nm. For a given common α-subunit the intensity of this band is correlated with the biological activity of the corresponding $\alpha\beta$-dimer. The spectrum of any dimer is distinctly different from the sum of α- and β-contributions, so supporting the concept that the subunit conformation in the dimer is different from that in the monomer.[526]

Analogues of angiotensin II with β-branched residues in position 5 possess spectra and biological properties identical to those of the native protein, confirming the influence of this residue on the organization of the Tyr^4 and His^5 site.[527]

The des(1–13) analogue of human β-endorphin, which does not possess opiate activity, binds to calmodulin with an associated increase in α-helicity and inhibits the calmodulin-mediated Ca^{2+} stimulation of cyclic nucleotide phosphodiesterase.[528]

In bradykinin-potentiating protein the 200 nm transition is only weakly affected by solvent or pH, but the tryptophan transition at 220–240 nm shows marked sensitivity to such changes. An enhancement of c.d. activity at 225 nm and $-100\,^{\circ}C$ is assigned to a change in conformational populations. The results of amino acid-substituted analogues were discussed in terms of the conformational stability of the molecule.[529]

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Nuclear Proteins. – Core-particle assembly, dissociation, and histone release from chromatin are all very fast processes occurring in less than 1 ms.[530] The aggregation states of nucleosomes and DNA-free histone octamers are not affected by the acetylation of up to 50% of the total lysine residues present. However, acetylation of $\geqslant 5$ lysine residues of each histone does lead to significant conformational changes within the histones, increased lability, and altered antigenic activity of histone H4.[531,532]

Synthetic chromatins produced by mixing core histones with synthetic polynucleotides have been studied by c.d. in order to measure their sensitivity towards temperature and ionic strength. A comparison of these results with those for natural systems highlights significant differences arising presumably from the unique nature of assembly in native systems.[533] *N*-Terminal oligopeptides derived from histone H2B possess an extended left-handed polyproline II helical structure the stability of which depends on chain length.[534]

Complex formation between actin and DNase I does not significantly alter the conformation of either of the proteins. U.v. c.d. studies indicate the absence of any aromatic residues on the interface formed between the two proteins.[535]

A small operator DNA fragment binds four *lac*-repressor headpiece proteins. The binding of the first pair of headpieces is specific and induces marked conformational changes. The binding of the second pair is similar to the non-specific binding to non-operator DNA. Addition of the core to the *lac* operator does not induce any conformational change. The relative roles of core and headpieces in the *lac*-operator interaction were discussed.[536] The secondary structure of this *lac*-repressor headpiece contains ~50% helix and some β-structure. The amino acid sequences responsible for these structures have been proposed.[537] The headpiece binds more strongly to double-stranded than to single-stranded polynucleotides.[538]

The binding constant for the interaction of the cyclic-AMP receptor protein with a synthetic DNA molecule, mimicking the *gal* operon, has been determined both in the presence and absence of cAMP. In both cases the protein-binding process induces a B–C transition.[539]

Membrane and Cell-surface Proteins. – Intact *Pencillium chrysogenum* mycovirus capsid protein contains 45% α-helix. Empty capsids, containing a protein of

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the same molecular weight as intact virus protein, contain 30% α-helix, suggesting that a conformational change occurs in the capsid on assembly with RNA, presumably *via* ionic interaction between protein and nucleic acid.[540]

Cardiac fatty-acid-binding proteins have been found to self-aggregate and to exist as at least four molecular species. A mathematical model has been formulated to allow quantitation of the various species in solution by deconvolution of the c.d. spectra. The results of fatty-acid-binding experiments and self-aggregation have led to the formulation of a theory of the importance of these phenomena to the activity of membrane-bound enzymes that are fatty acid or acyl-CoA dependent.[541]

On interaction with phosphatidylcholine, β-lactoglobulin undergoes a conformational change involving tryptophan residues, which leads to an increase in the α-helical content. The ability of the protein to interact with lipids is believed to be important to its transport through membrane systems.[542]

At acid pH lysozyme possesses an increased α-helical content over that present at neutral pH. This conformational change produces hydrophobic patches on the surface of the molecule, which allows increased interaction with phosphatidylcholine–cardiolipin vesicles. This pattern of behaviour is proposed to be common to other peripheral protein interactions with lipids.[543]

The structure of the water-soluble form of a plasma membrane proteolipid complex that forms a voltage-dependent cation channel contains 73% α-helix, 14% β-structure, and 12% random coil. Addition of SDS to the proteolipid complex has little effect on structure. Introduction of the protein into phosphatidylcholine–cholesterol liposomes leads to a small reduction in helical content and an almost total elimination of β-structure, presumably because of the formation of the cation channel structure in the interior of the protein.[544]

The predominant conformation of gramicidin A in dipalmitoylphosphatidylcholine liposomes is the antiparallel $\pi\pi_{LD}$ helix at protein/lipid ratios of $1:>300$, antiparallel β-sheet at 1 : 150, and a mixture of both of these at 1 : 10 to 1 : 30. In dimyristoylphosphatidylcholine liposomes the protein is predominantly in the antiparallel β-sheet form at all protein/lipid ratios. In the β-structure there appears to be a hairpin conformation with a DL turn between residues 6 and 7. These observations were discussed in light of various other studies on gramicidin conformation.[545]

A liposome-reconstituted porcine mitochondrial H^+-ATPase shows two negative bands at 208 and 219 nm in both the presence and absence of Mg^{2+} even though the presence of Mg^{2+} does induce an increase in α-helical content of the ATPase from 19 to 24%.[546]

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5 Magnetic Circular Dichroism

Contributed by T. Brittain and A. Mathews

General. – *Theory.* Group theoretical techniques have been used to analyse the geometrical dependence of magnetic circular dichroism (m.c.d.) spectra on the relative orientations of crystals, radiation, and external field for each point group. At arbitrary orientations the spectrum is a linear superposition of a definite number of irreducible spectra. M.c.d. has axial symmetry in the groups D_n, D_{nh}, C_{nv} ($n \neq 2$), D_{2n}, and D_{3d}.[547]

A theory has been developed of multi-photon m.c.d. for the two-identical-photon case. In addition to the normal A, B, and C terms, if in the two-photon case either the initial or the resonant state is degenerate, another additional transition-probability term occurs. Expressions for these terms and the associated bandshapes and temperature dependences have been derived using adiabatic approximations and a displaced harmonic-oscillator model.[548]

Expressions have been derived for the terms arising in magnetic vibrational c.d. Calculations on the coupling of ground electronic states with higher electronic states have been made in the case of B terms, and the possible utility of this form of spectroscopy in the elucidation of molecular structure has been pointed out.[549]

Instrumentation. The use of synchrotron radiation as a light source, particularly in the u.v. region, for m.c.d. studies has been described and holds the promise for rapid advances in future years.[550,551]

A number of spectrometers have been described that use various forms of light modulation to increase sensitivity. Performance characteristics are typically $\sim 2 \times 10^{-5}$ optical-density sensitivity in the region of 350–1000 nm.[552–554]

A procedure has been described to optimize experimental conditions in order to minimize artifacts sometimes observed in m.c.d. measurements. These anomalous spectral peaks occur mainly in the u.v. region and arise from magnetic-field inhomogeneity across the magnet bore, leading to site-dependent rotation of the monitoring light beam.[555]

Model Systems. A number of studies on model systems appropriate to haem-protein spectroscopy have been made. The first exception to the normal sign

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pattern $-+-+$ in the m.c.d. of haem has been reported in a series of tetrakis-(*O*,*O*′-dihalophenyl)porphyrins. The sign inversion in the Q bands as well as the normal pattern in the Soret bands ($+--+$) can be understood in terms of Michls perimeter model.[556] Zero-field splitting parameters have been determined for chloro(*meso*-tetraphenylporphinato)iron(III) using temperature-dependent m.c.d. measurements.[557] Tetrabenzoporphin-iron(III) has been shown to be high spin whereas its bisimidazole derivative is, as expected, low spin.[558] The temperature dependence of the m.c.d. spectra of high-spin Fe^{III} haem fluoride has been measured from the near-i.r. to the near-u.v. region. A model for this system leads to functions for the bands observed at 605 and 250 nm, which consist of equal amounts of π–π^* and charge-transfer character. The calculated vibronically induced intensity for the 0–1 components of these bands agrees with observations, and expressions are given for the m.c.d. of the vibronically induced bands.[559]

Proteins. – The use of m.c.d. in the analysis of metal-ion binding to proteins has been reviewed.[560]

Near-i.r. m.c.d. of metmyoglobin cyanide and fluoride, which are typical low- and high-spin Fe^{III} haem complexes, respectively, exhibits Faraday C terms and Faraday C and A terms in the 700–2000 nm region, respectively. These results are consistent with theoretical predictions which assume that the near-i.r. bands of Fe^{III} haem complexes are attributable to charge-transfer transitions from occupied a_1- and $a_{2\pi}$-orbitals to vacant Fe d_π-orbitals.[561] Sharanov and his co-workers have studied the origins of m.c.d. activity in both low- and high-spin ferrous haem proteins. Using low-temperature m.c.d. the origins of m.c.d. bands in both the visible and near i.r. have been assigned for a range of high-spin haem proteins and the analogous species produced by low-temperature photolysis of the corresponding CO complexes. These studies highlight the sensitivity of m.c.d. to the peculiarities of the haem-iron co-ordination sphere, provided by the various protein conformations. In particular it has been proposed that the position of the band maximum in the 690 nm region is directly correlated to the iron out-of-plane distance, whereas that at ~760 nm reflects the $Fe-N_y$ proximal (histidine) bond strength.[562,563] Although in the near-u.v. region the m.c.d. activity of low-spin Fe^{III} haem proteins is very similar, in the Q region marked differences are apparent between various proteins, particularly at low temperature. A theoretical model describing these

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findings has been derived and the role of π–d interactions in such proteins discussed.[564]

In a series of papers Thomson and co-workers have investigated the near-i.r. m.c.d. character of mammalian cytochrome *c* oxidase. In the oxidized cyanide-bound form of the enzyme a unique band at 1946 nm has been assigned to the existence of a ferromagnetically coupled, cyanide bridged, haem a_3–Cu_{a_3} pair.[565,566] Addition of NO to this system appeared to lead to binding to Cu_{a_3}, leaving the iron–copper pair still magnetically coupled *via* the CN^- bridge.[567] By deconvoluting the spectrum of the bisimidazole haem *a* spectrum from that of cytochrome oxidase the m.c.d. activity associated with Cu_a has been obtained. Comparison of the m.c.d. data derived in this way with those observed for the Cu centre in azurin shows that in the oxidase molecule the Cu centre exhibits 10–20-fold greater m.c.d. intensity. The Cu_a^{2+} centre is assigned d^9 rather than Cu^I–thiyl radial character.[568]

The overall general approach to the study of cytochrome *c* oxidase using m.c.d. has been reviewed.[569]

The assignment and polarizations of the near-i.r. charge-transfer transitions of low-spin metmyoglobin have been confirmed by m.c.d. measurements. The near-i.r. transition energies are markedly dependent on axial ligand and can easily distinguish, for example, the intrinsic phenolate ligand in catalase from the histidine in myoglobin.[570,571]

The intensity of the Soret m.c.d. spectra of various complexes of methaemoglobin and the α- and β-subunits correlates well with the spin state of the ferric haem.[572]

Cytochrome P450–CAM appears to possess an endogenous alcohol-containing amino acid or H_2O as the sixth ligand.[573,574] Replacement of the intrinsic ligand leads to two classes of complex. Addition of CO, NO, phosphine, nitrosoalkanes, and isocyanides yields the hyperporphyrin class. Complexes with σ-donors such as N and S ligands are of the non-hyperporphyrin class but still appear to retain

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the intrinsic cysteinate ligand.[575] M.c.d. of liposome-reconstituted cytochrome P450 and its reductase suggests that the two proteins, when reconstituted, are present as a complex for a significant percentage of the time.[576]

The ferric forms of L-Trp 2,3-dioxygenase and indoleamine 2,3-dioxygenase, at neutral pH, are high spin and mixed spin, respectively. Addition of Trp to these enzymes produces a different form of high-spin Trp dioxygenase and an increase in low-spin indoleamine dioxygenase. The sixth ligand appears to be water and histidine for the tryptophan and indoleamine oxygenases, respectively. The ferrous forms of both enzymes are typically high spin.[577] The ferric cyanide and CO complexes of both enzymes are similar to low-spin myoglobin. Addition of Trp to these forms produces a decrease in Soret m.c.d. activity. It is proposed that Trp binding bends the essentially linear Fe—C—N and Fe—C—O bonds.[578]

Glutamate-270 has been identified as playing a key role in stabilizing the kinetically important metal-co-ordinated water molecule in Co^{II}-carboxypeptidase A.[579] M.c.d. of reaction intermediates, trapped at low temperature, identified catalysis-related dynamic alterations in the active-site metal-co-ordination sphere of this enzyme.[580]

The m.c.d. spectrum of compound I of horseradish peroxidase is temperature independent down to 30 K. Below this temperature a phase change in the protein appears to increase the coupling between the $S = 1$ Fe^{IV} and the $S = \frac{1}{2}$ porphyrin π-cation radical and leads to a marked temperature dependence in the m.c.d. spectrum.[581]

At room temperature milk lactoperoxidase is high spin with a proximal histidine iron ligand and probably a carboxylate sixth ligand. At low temperature the enzyme converts to a low-spin form with an imidazolato sixth ligand.[582]

M.c.d. has been employed to obtain redox potentials for all the electron carriers in yeast complex III.[583]

At room temperature soybean leghaemoglobin exists as a mixture of high-spin and low-spin forms with His/H_2O and bisHis axial ligation, respectively. As this enzyme binds phenol to give a high-spin derivative, it is proposed that this complex may serve as a good model for other haem proteins with this type of axial ligation.[584]

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The m.c.d. spectrum of Hg,Cu metallothionein has been resolved into the contributions from the Cu thiolate and Hg thiolate groups.[585]

The m.c.d. properties of nitrogenase proteins have been reviewed.[586]

Oxidized ferredoxins I and II from *Desulfovibrio africanus* exhibit m.c.d. spectra characteristic of diamagnetic [4Fe–4S] clusters of 2+ oxidation state. Magnetization curves of reduced ferredoxin I give intercepts of 0.5 corresponding to a paramagnetic with $g \cong 2$ and $S = \frac{1}{2}$. This is taken to confirm the absence of other oxidized paramagnetic contaminants that normally interfere with spectral determinations of other $[4Fe–4S]^{1+ \text{ and } 2+}$ ferredoxins.[587]

6 Mössbauer Spectroscopy

Contributed by D. P. E. Dickson and S. H. Bell

During 1983 work using ^{57}Fe Mössbauer spectroscopy has been reported on haem proteins, iron–sulphur proteins, and iron-transport and -storage proteins. There has also been continuing interest in the use of Mössbauer spectroscopy to investigate protein dynamics. Huynh and Kent[588] have reviewed the use of Mössbauer spectroscopy in the study of biomolecules, and they have also considered the relationship with electron paramagnetic resonance measurements.

Haem Proteins. — The work on haem proteins falls into two main categories: the investigation of the simpler haem proteins and their reaction products and the study of the more complex haem proteins, enzyme systems, and model compounds, which frequently exhibit unusual spin and valence states of the iron atom.

Mössbauer measurements made on single-crystal samples, particularly in the presence of an applied magnetic field, can frequently yield more information than data obtained from the polycrystalline or frozen-solution samples, which are usually used in the case of Mössbauer investigations of biomolecules. Yakimov *et al.*[589] report a Mössbauer study on a single crystal of metmyoglobin at 4.2 K in a small applied magnetic field. The data show the sensitivity of the Mössbauer spectrum to the direction of the magnetic field and enable the direction of the haem plane to be determined with respect to the orientation of the crystal.

The possible existence of differences between the Mössbauer parameters of the haem iron atom in the α- and β-subunits of haemoglobin has been a subject of interest for some time. The current evidence is that any such differences are

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very small. Oshtrakh and Semenkin[590] report the results of a high-accuracy Mössbauer study of oxyhaemoglobin and deoxyhaemoglobin. The spectra were fitted to a superposition of two quadrupole-split doublet components with similar chemical shifts. The quadrupole splittings of the two components were found to be identical for deoxyhaemoglobin but different for oxyhaemoglobin, reflecting a difference in the inequivalence of the Fe^{2+} sites in the α- and β-subunits in these two forms of the protein.

The effect of radiation and other treatments on haemoglobin is of both medical and biochemical interest. It has been shown that exposure of rat haemoglobin to microwave radiations causes the appearance of an additional component in the Mössbauer spectrum.[591] Chevalier *et al.*[592] report a Mössbauer study of the effects of *X*-radiation on both oxy and deoxy forms of haemoglobin in a wide range of doses. Large differences were observed, with deoxyhaemoglobin dissociating to form only one non-haem ferric compound while oxyhaemoglobin produces this same compound as a last step after intermediary production of deoxyhaemoglobin, methaemoglobin, haemichromes, and haemochromes. The results of heat treatment are compared with the data from this *X*-radiation study, and some explanations for the formation of the different compounds are given based on structural considerations.

Many enzyme systems contain a number of haem iron sites with various oxidation and spin states for the iron atom, depending on the redox level of the enzyme. Mössbauer spectroscopy provides an important means of investigating these states. Kent *et al.*[593] have obtained Mössbauer spectra for samples of cytochrome *c* oxidase obtained from beef heart and therefore containing ^{57}Fe with the 2% natural abundance. The spectra were found to be very similar to those of the *a* and a_3 sites of cytochrome c_1aa_3 from *Thermus thermophilus*. Thus, many of the conclusions derived from studies of the bacterial enzyme, which is available enriched with ^{57}Fe, also apply to the mammalian enzyme. The Mössbauer data indicate that the a_3 iron site in the native enzyme, as isolated, is high-spin ferric and is spin-coupled to Cu^{2+}, as has been previously suggested. The broad lines observed in the spectra indicate that the a_3 site in the native enzyme is heterogeneous. The reduced a_3 site has Mössbauer parameters consistent with a high-spin ferrous assignment. The Mössbauer data on the reduced cytochrome *a* site suggest that the iron is in a low-spin ferrous state similar to that of cytochrome b_5 and cytochrome *c*.

Possible sources of model compounds for the iron sites in haem proteins and enzymes are iron–porphyrin complexes, polypeptides produced either synthetically or derived from haem proteins, and various other organo-iron complexes. Lukas *et al.*[594] report a Mössbauer spectroscopic investigation of a large number

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of metal protporphyrin-iron(III) complexes. Peterson *et al.*[595] have derived a number of polypeptides from horse-heart cytochrome *c* by proteolytic digestion. Mössbauer measurements on these show that the nonapeptide and the undecapeptide may be useful models for the iron environment in ferricytochrome *c*. Boso *et al.*[596] have investigated oxidized chloro-5,10,15,20-tetra(mesityl)-porphyrinato-iron(III) as a possible model complex for derivatives of horseradish peroxidase and cytochrome *c* peroxidase. It was found, however, that the magnetic features in the Mössbauer spectra are markedly different. These differences are interpreted in terms of the strength of the axial crystal field and spin coupling within the complexes. A possible new model compound for the active centre of oxycytochrome P450 has been reported.[597,598] Mössbauer data on this compound suggest that an axial cysteinyl sulphur atom is a ligand for iron in this haem protein. This is an interesting result in that cysteinyl sulphur ligands are usually considered to be a feature of the iron–sulphur proteins, which are a quite different class of iron-containing proteins from the haem proteins. Of related interest is the discovery of an enzyme containing both haem and iron–sulphur centres within a single molecule, which is discussed in the next section.

Iron–Sulphur Proteins. — Mössbauer spectroscopy has provided an important technique for the investigation and identification of the various types of iron–sulphur clusters found in iron–sulphur proteins and enzyme systems. Typical of work of this kind is the investigation of the soluble ferredoxin from *Thermus thermophilus*, which has been examined by Mössbauer spectroscopy and electron paramagnetic resonance and shown to contain one [3Fe–*x*S] cluster and one [4Fe–4S] cluster.[599] Emptage *et al.*[600] have shown that during activation of the enzyme aconitase Fe^{2+} is incorporated into a [3Fe–4S] cluster to yield a structure with a [4Fe–4S] core. Mössbauer spectroscopy shows that one of the iron atoms in this [4Fe–4S] cluster is drastically altered in the presence of citrate, and this is the iron atom acquired during activation. In the reduced $[4Fe–4S]^{1+}$ state of the cluster, this atom becomes essentially high-spin ferrous. The dramatic increase in the chemical shift of this atom on reduction suggests that the ligand environment of this iron site is five co-ordinate and that the cluster may act as a Lewis acid.

Hydrogenases are enzymes containing iron–sulphur centres that catalyse the terminal steps of hydrogen evolution and utilization in a number of micro-organisms. Teixeira *et al.*[601] have investigated the redox properties of the nickel

[595] J. Peterson, M. M. M. Saleem, J. Silver, M. T. Wilson, and I. E. G. Morrison, *J. Inorg. Biochem.*, 1983, **19**, 165.

[596] B. Boso, G. Lang, T. J. McMurray, and J. T. Groves, *J. Chem. Phys.*, 1983, **79**, 1122.

[597] M. Schappacher, L. Ricard, R. Weiss, R. Montiel-Montoya, U. Gonser, E. Bill, and A. X. Trautwein, *Inorg. Chim. Acta*, 1983, **78**, L9.

[598] L. Ricard, M. Schappacher, R. Weiss, R. Montiel-Montoya, E. Bill, U. Gonser, and A. X. Trautwein, *Nouv. J. Chim.*, 1983, **7**, 405.

[599] R. Hille, T. Yoshida, G. E. Tarr, C. H. Williams, M. L. Ludwig, J. A. Fee, T. A. Kent, B. H. Huynh, and E. Munck, *J. Biol. Chem.*, 1983, **258**, 13 008.

[600] M. H. Emptage, T. A. Kent, M. C. Kennedy, H. Beinert, and E. Münck, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 4674.

[601] M. Teixeira, I. Moura, A. V. Xavier, D. V. Dervartanian, J. Legall, H. D. Peck, B. H. Huynh, and J. J. G. Moura, *Eur. J. Biochem.*, 1983, **130**, 481.

and iron–sulphur centres in the hydrogenase from *Desulfovibrio gigas*, mainly by electron paramagnetic resonance. Their preliminary Mössbauer measurements indicate the presence of a [3Fe–*x*S] cluster and two [4Fe–4S] clusters.

Nitrogenases are enzymes consisting of a number of proteins that contain iron–sulphur clusters, both of the [4Fe–4S] type as well as of a much more complex type, containing molybdenum as well as iron, which has not been well characterized yet and remains the subject of considerable interest. A review of Mössbauer studies of nitrogenase has been given by Zimmermann and Trautwein,[602] and this concentrates on the information on the molybdenum iron protein. The [4Fe–4S] clusters in nitrogenase are thought to be rather different from those in normal ferredoxins, and Mössbauer investigations of certain [4Fe–4S] model compounds have been undertaken to shed light on these differences.[603] Two classes of model compounds were found with different iron ligands. In one type there appears to be charge delocalization over the four-iron cluster, while in the other type there is charge localization onto pairs of iron atoms within the cluster. A Mössbauer study of single and double [Mo–3Fe–4S] clusters has been undertaken in order to investigate possible model compounds for the cofactor centre of the molybdenum iron protein of nitrogenase.[604] The data indicate that some of the clusters have an $S = \frac{3}{2}$ state as found in the cofactor, and which appears to be important in identifying suitable models.

The enzyme sulphite reductase from *Escherichia coli* and its complexes with carbon monoxide and cyanide have been investigated by Mössbauer spectroscopy.[605,606] The subunit of the enzyme has been shown to contain a sirohaem exchange-coupled to a [4Fe–4S] cluster. The existence of a haem iron associated with the iron–sulphur cluster is a novel feature of this enzyme. The complex enzyme undergoes a one-electron reduction in which the haem iron is reduced from a low-spin ferric state to a low-spin ferrous state with the [4Fe–4S] cluster in the 2+ oxidation level. A further one-electron reduction reduces the [4Fe–4S] cluster. Analysis of the Mössbauer spectra of the fully reduced complexes indicates that the iron atoms in the [4Fe–4S] cluster are inequivalent in pairs, one pair having a more ferric character and the other pair a more ferrous character.

Photosynthetic reaction centres are a complex enzyme system containing iron–sulphur clusters. Mössbauer studies have been carried out on the photosynthetic reaction centres in enriched subchromatophore fragments of *Rhodopseudomonas spheroides*.[607] The spectra show two quadrupole-split

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[603] A. Simopoulos and A. Kostikas, *Sol. Energy R. and D. Eur. Community, Ser. D*, 1983, **2**, 239.

[604] P. K. Mascharak, G. C. Papaefthymiou, W. H. Armstrong, S. Foner, R. B. Frankel, and R. H. Holm, *Inorg. Chem.*, 1983, **22**, 2851.

[605] J. A. Christner, E. Münck, P. A. Janick, and L. M. Siegel, *J. Biol. Chem.*, 1983, **258**, 11 147.

[606] J. A. Christner, P. A. Janick, L. M. Siegel, and E. Münck, *J. Biol. Chem.*, 1983, **258**, 11 157.

[607] N. Y. Uspenskaya, A. A. Novakova, A. Y. Aleksandrov, R. N. Kuzmin, A. A. Kononenko, and A. B. Rubin, *Biofizika*, 1983, **28**, 376.

doublet components, one with parameters indicative of a high-spin ferrous iron atom, the other with parameters similar to those of a ferredoxin. On reduction with sodium dithionite the high-spin ferrous component is reduced to a high-spin ferrous state.

Iron-transport and -storage Proteins. – Enterobactin is one of the group of compounds, some of which are proteins, that are responsible for the chelation and take-up of iron by living organisms. Pecoraro *et al.*[608] report a Mössbauer study of ferric enterobactin as a function of pH. Their data show the importance of pH in relation to the iron binding.

Iron metabolism in living systems and the transfer of iron between storage, transport, and other forms are of considerable interest, particularly in relation to physiological and medical factors. Mössbauer spectra have been obtained from certain membranes within the human placenta and have been compared with those of the human iron-storage and -transport proteins ferritin and transferrin.[609] The data indicate that the majority of the iron in the membranes is in the same form as that in ferritin.

Protein Dynamics. – In recent years Mössbauer spectroscopy has developed an important role in relation to the study of living systems as a result of its sensitivity to any motion of the Mössbauer nucleus. Mössbauer information on the dynamics of proteins as a function of temperature has shown many interesting features. Nowik *et al.*[610] discuss a number of recent Mössbauer studies of ^{57}Fe in a variety of biological systems, including proteins and membranes. The absorption spectra consist of a narrow part attributed to elastic processes and a wide part attributed to inelastic processes. These features are interpreted by a model of bounded diffusion in which overdamped harmonically bound particles are in Brownian motion. Excellent fits to the experimental data can be obtained; these yield values of the mean-square displacements and the diffusion coefficients of the part of the system containing the ^{57}Fe nuclei. A related interpretation of the same type of data by Knapp *et al.*[611] treats the Mössbauer spectra as arising from a minimum of three Brownian oscillator modes. Two of these modes are extremely overdamped and the third has a diffusion-like character. The drastic changes of the Mössbauer spectra with temperature are simulated by an extended version of the Brownian oscillator. With this oscillator the protein is characterized by conformational substates and transition states, with the former responsible for the solid-like behaviour and the latter responsible for the liquid-like behaviour.

A Mössbauer study of the dynamics of the haem iron in metmyoglobin and deoxymyoglobin crystals over a wide range of temperatures has been carried out

[608] V. L. Pecoraro, G. B. Wong, T. A. Kent, and K. N. Raymond, *J. Am. Chem. Soc.*, 1983, **105**, 4617.
[609] S. H. Bell, P. J. Brown, D. P. E. Dickson, and P. M. Johnson, *Biochim. Biophys. Acta*, 1983, **756**, 250.
[610] I. Nowik, S. G. Cohen, E. R. Bauminger, and S. Ofer, *Phys. Rev. Lett.*, 1983, **50**, 1528.
[611] E. W. Knapp, S. F. Fischer, and F. Parak, *J. Chem. Phys.*, 1983, **78**, 4701.

by Bauminger *et al.*[612] Above a critical temperature of 220 K there are two kinds of thermal fluctuations present: a fast fluctuation associated with a steep decrease of the total resonant absorption with increasing temperature and a slower fluctuation associated with bounded diffusion motion. By using both discrete-jump and continuous-diffusion models the essential parameters of the iron motion, *i.e.* the mean-square displacements and the diffusion coefficients, are obtained. Although the same general kind of behaviour is obtained in both proteins, there are significant differences suggesting that the main dynamic phenomena observed reflect large-scale internal motions of the protein.

Mayo *et al.*[613] have obtained Mössbauer data on frozen solutions of haemochromes and of human deoxyhaemoglobin selectively enriched in either the α- or β-chains. Large differences in the mean-square displacements of the iron atom at temperatures above 210 K were observed, demonstrating that when iron is bound to a rigid part of the protein, as in haemochrome, certain motions are suppressed. Comparison of the motions of these two systems proves that molecular diffusion can be neglected in an analysis of the dynamics at around 250 K.

The Mössbauer study of photosynthetic reaction centres, discussed above,[607] also included measurements of the temperature dependence of the spectra, which were analysed to give information on the dynamics of the system. This type of analysis may be possible in many Mössbauer spectroscopic studies of biological systems and could frequently enhance the amount of information that can be obtained.

7 Interacting Protein Systems

Contributed by L. W. Nichol, D. J. Winzor, and P. D. Jeffrey

A notable feature of the work this year has been the number of investigations concerned with methodological aspects of the study of protein interactions. Some of these are highlighted as a prelude to the development of associated themes in subsequent sections.

Contributions to Methodology. – In an attempt to explain the unusual electrophoretic behaviour of fish-muscle creatine kinase, a phenomenological theory of transport of reacting systems has been formulated for moving-boundary electrophoresis of a sulphydryl protein undergoing oxidation in the presence of a gradient of molecular oxygen.[614] Numerical simulation of mass migration has also been used to examine the effect of microheterogeneity on the sedimentation behaviour of self-associating proteins, with a view to determining the extent to which such inhomogeneity of a solute is likely to affect the shapes of

[612] E. R. Bauminger, S. G. Cohen, I. Nowik, S. Ofer, and J. Yariv, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 736.
[613] K. H. Mayo, D. Kucheida, F. Parak, and J. C. W. Chien, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 5294.
[614] J. R. Cann, N. H. Fink, and D. J. Winzor, *Arch. Biochem. Biophys.*, 1983, **221**, 57.

sedimentation-velocity patterns for rapidly self-associating proteins.[615] In that regard criteria have been established to determine whether a bimodal schlieren pattern reflects self-association for which the rate of re-equilibration is slow, rapid, or rapid but ligand-mediated, behaviour illustrated with sedimentation-velocity studies of tubulin association.[616] Simulated examples and experimental results with β-lactoglobulin A have been employed to test the feasibility of combining results from sedimentation-velocity and sedimentation-equilibrium experiments on the same self-associating system in order to evaluate the sedimentation coefficients of the associating species and the *s-c* dependence coefficient.[617] Developments in the prediction of sedimentation coefficients from geometrical considerations have included introduction of the tri-axial ellipsoid as a hydrodynamic shape[618] and the derivation of new hydrodynamic interaction tensors that provide an improved means of computing frictional coefficients of objects describable by a surface shell of many small spheres.[619]

A light-scattering study of the effect of poly(ethylene glycol) on the thermodynamic activity of bovine serum albumin has yielded results in good agreement with the predictions of excluded-volume theory,[620] which also provides a satisfactory molecular basis for the exclusion of proteins by loose connective tissue (human umbilical cord).[621] In a study of the influence of poly(ethylene glycol) on the properties of skeletal-muscle actin, the inert polymer was shown to accelerate the rate and to increase the extent of actin polymerization, the effects again being consistent with predictions based on the excluded-volume concept applied for the first time to the effects of a space-filling macromolecule on an indefinitely self-associating system.[622] On the other hand, the protein-stabilizing action of small inert molecules and their enhancing effect on protein associations are considered in terms of preferential hydration,[623,624] a concept that has also been invoked to account for the enhanced interaction of oxygen with haemoglobin in the presence of concentrated ethylene glycol.[625] Other thermodynamic studies have involved consideration of equilibrium partitioning of concentrated protein solutions in exclusion chromatography[626] and investigations of the partitioning of proteins in the aqueous Ficoll–dextran biphasic system.[627,628] A simple method based on equilibrium dialysis and ultrafiltration

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[617] J. M. Beckerdite, C. A. Weirich, E. T. Adams, jun., and G. H. Barlow, *Biophys. Chem.*, 1983, **17**, 203.
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[620] D. Knoll and J. Hermans, *J. Biol. Chem.*, 1983, **258**, 5710.
[621] F. A. Meyer, *Biochim. Biophys. Acta*, 1983, **755**, 388.
[622] R. L. Tellam, M. J. Sculley, L. W. Nichol, and P. R. Wills, *Biochem. J.*, 1983, **213**, 651.
[623] T. Arakawa and S. N. Timasheff, *Biochemistry*, 1982, **21**, 6536.
[624] T. Arakawa and S. N. Timasheff, *Arch. Biochem. Biophys.*, 1983, **224**, 169.
[625] R. N. Haire and B. E. Hedlund, *Biochemistry*, 1983, **22**, 327.
[626] A. P. Minton, *Biophys. Chem.*, 1983, **18**, 139.
[627] B. Y. Zaslavsky, N. M. Mestechkina, L. M. Miheeva, and S. V. Rogozhin, *J. Chromatogr.*, 1983, **256**, 49.
[628] B. Y. Zaslavsky, N. M. Mestechkina, and S. V. Rogozhin, *J. Chromatogr.*, 1983, **260**, 329.

has been described[629] for determining the extent to which the hitherto assumed concept of charge conservation is a valid approximation in studies of protein–protein and protein–ligand interactions.

In regard to methodological aspects of ligand-binding measurement, a procedure employing a double conical gel-permeation column has been presented[630] as an alternative to the rate-of-dialysis method for determining an entire ligand-binding curve with a single sample of protein solution. The empiricism associated with rate-of-dialysis studies of ligand binding has now been removed by identification of the principles involved as those inherent in the measurement of diffusion coefficients by the diaphragm cell method.[631] A note of caution has been expressed in relation to the quantitative assessment of protein–ligand interactions by fluorescence measurements, where absorption of the excitation light by the sample can lead to substantial systematic errors in the equilibrium constants so determined.[632]

Analytical expressions have been derived for the ionic-strength dependence of the electrostatic interaction energy between two charged proteins and hence of the rate constant of reaction between them, application of the theory being illustrated with results for the interactions of cytochrome *c* with cytochrome b_5 and cytochrome oxidase.[633] In other studies concerned with reaction rates, the effect of viscosity on the dissociation of enzyme–ligand complexes has been formulated in theoretical terms,[634] and a theoretical study has been made of the length dependence of rate constants for end-to-end association and dissociation of linear aggregates.[635] Fluorescence polarization has been used to comment upon the dissociation of lipoic acids from and their rotation between binding sites on the α-ketoglutarate dehydrogenase complex from *Escherichia coli*.[636] Electron spin resonance spectroscopy and stopped-flow methods have established that the rate of interaction between the cAMP-dependent protein kinase catalytic subunit and its substrate, histone H1, is close to the rate of phosphate transfer from the phosphoenzyme intermediate to protein substrate.[637]

Ligand Binding and Allosteric Effects. – *Thermodynamics and Conformational Changes.* A theoretical analysis of binding equations was conducted in order to probe the magnitude of thermodynamic non-ideality effects in terms of composition-dependent activity coefficients assessed on a statistical-mechanical basis of excluded volumes.[631] Aided by equilibrium dialysis and frontal gel-chromatography results on the binding of tryptophan and of Methyl Orange

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[630] J. T. Wong and L. S. Porter, *Anal. Biochem.*, 1983, **130**, 491.
[631] C. L. Ford, D. J. Winzor, L. W. Nichol, and M. J. Sculley, *Biophys. Chem.*, 1983, **18**, 1.
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[633] J. V. Van Leeuwen, *Biochim. Biophys. Acta*, 1983, **743**, 408.
[634] G. R. Welch, B. Somogyi, J. Matkó, and S. Papp, *J. Theor. Biol.*, 1983, **100**, 211.
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to bovine serum albumin, studied over a wide range of concentration, a reassurance was provided that effects of non-ideality, including a predicted acceptor-concentration dependence of the binding function, were of small magnitude.[631] On the other hand, a similar approach using excluded-volume calculations showed that non-ideality effects were responsible for an observed increase in the maximal velocity for the catalytic reduction of pyruvate by lactate dehydrogenase resultant on the introduction of inert macromolecules acting as space-fillers.[638] Among other thermodynamic considerations of ligand binding, particular emphasis has been given to estimation of enthalpy changes,[639] such as in the calorimetric study of the binding of indole and phenylethane boronic acid to chymotrypsin,[640] in the interaction of hormones and neuropeptides with calmodulin,[641] in the association of saccharides with *Helix pomatia* A haemagglutinin,[642] and in the co-operative oxygenation of dimeric and tetrameric haemoglobins.[643] Models involving a variety of reactions coupled to protein–ligand interactions have been formulated that can account for heat-capacity changes and enthalpy–entropy compensation apparent, for example, in the binding of nucleotides to ribonuclease A.[644] Some relatively unusual adsorption methods, in addition to affinity chromatography,[645] were described to investigate specific ligand binding, notably the ammonium sulphate-assisted adsorption of carbohydrate-binding proteins on cellulose ester filters[646] and the transfer of subunits of acetylcholine receptor in a renaturing environment to nitrocellulose sheets followed by binding of a radioactively labelled ligand monitored by autoradiography.[647]

There is continuing evidence for significant changes in protein structure induced by ligand binding. Small-angle *X*-ray scattering was used to show that the radius of gyration of lobster arginine kinase, composed of a single polypeptide chain, decreased by approximately 0.12 nm on binding ADP–Mg or ATP–Mg,[648] while studies on villin, an F-actin-binding protein with at least three Ca^{II}-binding sites,[649] revealed that the axial ratio of the equivalent prolate ellipsoid increased from 4.5 to 8 on binding calcium ions.[650] Considerably more detail on the stereochemistry of protein–ligand interactions is being provided by *X*-ray crystallographic and n.m.r. studies, exemplified by the finding that NAD binding likely gives rise to sequential structural changes of the tetramer of

[638] L. W. Nichol, M. J. Sculley, L. D. Ward, and D. J. Winzor, *Arch. Biochem. Biophys.*, 1983. **222**, 574.
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glyceraldehyde 3-phosphate dehydrogenase[651] and by the use of transferred n.O.e. measurements to elucidate glycosidic bond conformations in complexes of coenzyme analogues and dihydrofolate reductase.[652]

Allosteric Effects. In the theoretical area of the mathematical description of non-hyperbolic binding curves two major trends have been observed. The first involves attempts to reduce the number of parameters required to define the system from the number conventionally needed to interpret multiple binding of a ligand to a non-associating acceptor in terms of either the Monod–Wyman–Changeux or the Koshland–Némethyl–Filmer models. Thus, it has been suggested on the basis of empirical relations between the three MWC parameters that, in effect, haemoglobin oxygenation can be described by one adjustable parameter,[653] and further comment has been made on the value of the Hill coefficient as a convenient measure of co-operativity, the coefficient being expressable in terms of the roots of a polynomial describing the binding response.[654] In the same vein, Bardsley and Wright[655] discussed the extent of sigmoidicity in kinetic or binding curves in terms of two parameters, one measuring the fraction of the vertical span of the curve that is sigmoid and the other measuring the S-shaped character in the sigmoid region. The second development of possibly greater future potential stems from the ability to monitor, by techniques such as n.m.r. spectroscopy, ligand binding at individual sites on the acceptor; the theoretical work of Ackers and co-workers[656] on providing relationships between microscopic binding energies and equilibrium constants for a two-co-operative-site model is noteworthy in this respect. Mention should also be made in the theoretical domain of further work on allosteric enzymic systems where the substrate and an inhibitor compete for the same sites on the acceptor[657] and the possibility of determining thermodynamic allosteric parameters of an enzyme undergoing steady-state turnover.[658]

In parallel with theoretical advances, there have been many experimental studies that implicate co-operative interactions between ligand-binding sites on particular proteins. Comment has been made on the asymmetry of the sigmoidal binding response of the oxygen–haemoglobin system, which appears possible only if the α–α and β–β subunit interactions change by different amounts on oxygenation;[659,660] hydrogen-exchange studies have located seven amide NH protons around two turns of a helical segment in the haemoglobin β-chain, which exchange 30-fold faster in the oxygenated form than in the deoxy

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[659] G. Weber, *Nature (London)*, 1982, **300**, 603.
[660] L. Peller, *Nature (London)*, 1982, **300**, 661.

state.[661] A two-state model was used to analyse oxygen binding to *Panulirus interruptus* haemocyanin[662] and to tubular polymers formed after limited trypsinolysis of native cylindrical *Helix pomatia* β_c-haemocyanin.[663] Reports were also presented of co-operative interactions between the drug-binding sites on calmodulin,[664] the calcium- and cadmium-binding sites on troponin C,[665] and effector-binding and catalytic sites on a variety of enzymes, including NAD malic enzyme,[666,667] fructose 1,6-bisphosphatase,[668,669] adenylate cyclase,[670] hexokinase II,[671] and aspartate transcarbamoylase derivatives containing Ni^{II} or Co^{II} in place of the endogenous zinc ion bound to each of the regulatory subunits.[672] Of particular interest in the latter study was the use of Ni^{II} as a spectral probe for measuring structural alterations at the intersubunit-binding domains induced by binding substrate, bisubstrate, and a substrate analogue.[672] The freezing of a particular conformation state of *Acinetobacter* citrate synthase by chemical crosslinking with a bifunctional reagent was also noteworthy since it eliminated pre-existing co-operative interactions between regulatory sites, an effect reversed by cleavage of the crosslinks.[673]

Interplay Between Ligand Binding and Protein Association. In addition to allosteric effects or concomitant with them, the preferential binding of ligand to different association states of an acceptor and the initiation of an association by the ligand are also underlying causes for deviations of a binding response from hyperbolic form.[674] This theme was developed in a theoretical framework by Kurganov,[675] who considered a monomer–dimer enzymic system in which substrate is bound in the region of monomer contact, thereby perturbing the equilibrium in favour of dimer. It found experimental illustration in work on phosphofructokinase,[676,677] where it was shown that fructose 2,6-bisphosphate, a likely key intermediary in the regulation of carbohydrate metabolism in the liver, promotes association of the enzyme in a way that may well operate *in vivo*.

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Similarly, the self-associations of acetyl-CoA carboxylase[678] and glycogen phosphorylase *b*[679] were found to be promoted by citrate and AMP, respectively. There was direct sedimentation-velocity and -equilibrium evidence that chorismate mutase/prephenate dehydrogenase associated to a tetrameric form on the binding of NAD or tyrosine, which inhibits both activities of the bi-functional enzyme in the presence of NAD.[680] In the latter work, results were analysed in terms of a dimer–tetramer model with preferential binding of NAD and tyrosine to the tetramer, the conceptual link thereby being made with the work of Kurganov[675] and earlier formulations.[674]

Earlier theory had also encompassed the possibility of ligand-promoted dissociation, exemplified by the preferential binding of monosaccharides to the monomer of galactose- and maltose-binding proteins, both monomer–dimer systems.[681] In addition, further comment was made on the regulatory role of the dissociation of glyceraldehyde 3-phosphate dehydrogenase induced by substrates.[682]

Self-associations. – *Dissociation and Reassembly of Subunits.* The assembly of aspartase transcarbamoylase from its catalytic and regulatory subunits[683] provides one example of investigations of subunit interactions operating in a diverse range of systems. A study of the size and shape of the glucocorticoid receptor from the mouse has indicated that the inert form is probably tetrameric and that dissociation into subunits may provide the mechanism of activation.[684] Brain spectrin hybrid tetramers may be dissociated into subunits and reassociated with resumption of activity, and hybridization experiments have revealed that brain and erythrocyte spectrins belong to a closely related family of proteins.[685] Dissociation and reassociation experiments with the 16-membered ribulose 1,5-bisphosphate carboxylase complex have shown that reactions involving nucleotides, Mg^{II}, and several intermediates account at least partly for the light dependence of its assembly.[686] Experiments with another hybrid enzyme, human-liver alcohol dehydrogenase, have led to the conclusion that the ten isoenzymes identified arise from four different subunits in six heterodimers and four homodimers.[687] Information about the rates characteristic of such processes has been derived from small-angle *X*-ray scattering studies of CO-liganded haemoglobin subunits for which the half-time governing tetramer formation is about 12 s.[688] A light-scattering study of the effect of salts and ureas on the dissociation of concanavalin A tetramers to dimers has suggested that polar and

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ionic forces are the most important in maintaining the tetrameric structure.[689] The same conclusion was reached concerning the forces stabilizing the hexamers of *Homarus americanus* haemocyanin.[690] A major contribution to knowledge of the architecture of hybrid haemocyanin complexes was made by Lamy and co-workers[691,692] in studies of *Limulus polyphemus* oligomers by electron-microscope image-reconstruction and computer-imaging techniques, in conjunction with results of much previous work. The effects of metal ions on the assembly and co-operative ligand binding of arthropod haemocyanins also received attention.[693,694] In the histone field, it is still not clear whether the octamer is constituted as $[(H2A\text{-}H2B)(H3\text{-}H4)]_2$ or $(H2A\text{-}H2B)(H3)_2(H4)_2(H2A\text{-}H2B)$. Thus, ultracentrifugal studies of dissociation and reassociation[695] were interpreted in terms of the former, whereas the results of other reconstitution experiments were consistent with an octamer which included an $(H3)_2(H4)_2$ tetramer.[696,697]

Homogeneous Self-associations. Techniques of chemical modification,[698] laser-light scattering,[699] and spin-label electron paramagnetic resonance[700] have been used to study the interaction of haemoglobin S within the erythrocyte or with the erythrocyte membrane. Similarly, a variety of techniques has been used both to identify new protein self-associations and to throw some light on their possible biological functions. For example, it has been found that aggregation of cardiac fatty-acid-binding protein is responsible for the complex binding curve for fatty acid,[701,702] that the self-association of sarcoplasmic reticulum adenosine-triphosphatase produces a dimer that might be the vesicular form of the protein,[703] and that the indefinite self-association of α-ketoglutarate dehydrogenase complex may provide the mechanism that stabilizes the complex by hydrophobic interactions.[704] Experiments with glucose 6-phosphate dehydro-

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genase in human erythrocytes showed that dissociation to inactive monomers is not responsible for its lowered activity *in vivo*.[705] On the other hand, in yeast hexokinase, the monomer does seem to be the catalytically important form.[706] Other self-associating systems explored include secretin,[707] malate synthase,[708,709] and procollagen IV, where disulphide bridging stabilizes tetramers formed in a spontaneous and reversible equilibrium.[710] A very interesting sedimentation-velocity study of a human leukocyte interferon produced by recombinant-DNA technology revealed that the protein is self-interacting,[711] but a more critical investigation is required to establish the pattern and the thermodynamic parameters of the self-association.

Tubulin and Microtubules. Circular-dichroic spectral studies have indicated that the formation of microtubules is better described by a hybrid model involving direct incorporation of oligomeric species rather than by exclusive condensation polymerization of tubulin dimer.[712] Much information about structural transitions during the assembly and disassembly process was gained by time-resolved synchrotron *X*-ray scattering.[713] In another assembly mode, tubulin sheets can be formed in the presence of zinc ions: comparison of the electron micrographs of sheets formed in the presence and absence of microtubule-associated proteins (MAPs) suggests that MAPs lie in the groove between protofilaments and bind to several tubulin dimers.[714] In another study it was concluded that zinc ions stimulate tubulin assembly by binding directly to the protein, rather than *via* a ZnGTP complex.[715] Cibacron Blue[716] and S-100 protein[717] were found to inhibit tubulin assembly, a possible physiological role for the latter being suggested in the assembly–disassembly of microtubules. The tubulin–colchicine system continues to be the subject of much work and has been described in terms of a model involving the copolymerization of tubulin and tubulin–colchicine complex.[718,719] In contrast, another study[720] has favoured the proposition that the overall polymerization in the presence of tubulin–colchicine is described by competition between an intrinsically unaltered nucleation process and the process of propagation inhibited by the

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binding of tubulin-colchicine to the microtubule ends. It is also possible that microtubule inhibition arises from distortion of the normal protomer bonding geometry by the drug.[721]

Mixed Associations. – *Reactions Between Dissimilar Proteins.* A strong impression is gained from the literature of the ingenious deployment of an increasingly sophisticated battery of techniques to detect and to characterize heterogeneous association. Methods have included fluorescence spectroscopy to study the interaction between aldolase and glycerolphosphate dehydrogenase,[722] *X*-ray crystallography for examination of the kallikrein A-trypsin inhibitor complex,[723] sedimentation equilibrium in the airfuge to investigate the concanavalin A–serum glycoprotein systems,[724] and the use of structural probes.[725] In the latter study, chemical modification of cytochrome *c* oxidase indicated that Asp-112, Glu-114, and Glu-198 are involved in the binding to cytochrome *c*. In the same area, it has been shown that the interaction of cytochrome *c* with blue-copper proteins, azurin and plastocyanin, involves the same positively charged surface on cytochrome *c* that participates in other redox reactions, even though these blue-copper proteins bear opposite charge in their interaction domains.[726] Studies of the interaction of cytochrome P450 with NADPH-cytochrome P450 reductase support the proposal of formation of a 1:1 complex in liposomal membranes.[727] With respect to proteinase inhibitors, studies have been made of the interaction between α_2-macroglobulin and various serine proteases.[728,729] In addition, elucidation of the interaction of the purified rabbit-bone collagenase and its inhibitor has strongly suggested that latent collagenase should not be identified with this complex.[730]

Mixed associations have also arisen in a variety of other areas, including the use of concanavalin A[731] and monoclonal antibodies[732–736] to probe topo-

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graphical features of macromolecular surfaces and cells. A most interesting example of indefinite heterogeneous association involving a tetrameric protein, concanavalin A, and a multivalent ligand, dextran, was provided in a light-scattering study of the time-course of precipitin reactions.[737] Such reversible crosslinking reactions paralleling those in antigen–antibody systems may well be encountered more frequently in the future.

Insulin Receptor. Radiation-inactivation studies yielded information about the putative interaction between the two components for a model of the receptor involving a binding component ($M_r \sim 100\,000$) and an affinity-regulatory component ($M_r \sim 300\,000$).[738] The production of monoclonal antibodies to the human insulin receptor also promises to provide new insights into its structure.[739] The involvement of disulphide interchange in the mechanism of interaction of insulin and its receptor gained further support with findings that indicated that receptor sulphydryl groups are required for insulin action and their interchange with insulin is needed to activate post-receptor pathways[740] and that insulin is involved in interactions between receptor subunits by a process involving disulphide–sulphydryl exchange.[741] An interesting sidelight was the use of insulin binding to cellular receptors as an indicator of the effects of high temperature on cells, or more particularly on membrane proteins.[742] Work on post-binding events included evidence for internalization of one of the subunits in rat adipocytes following binding of labelled insulin.[743] In addition, much progress has been made in the understanding of the interplay between insulin binding and phosphorylation of receptors. Reports have included the identification of kinase and phosphatase activities in human placenta,[744] the finding that insulin stimulates phosphorylation of both α- and β-subunits of the receptor,[745] the idea that the phosphorylation occurs by activation of the kinase activity, which appears to be tightly associated with the receptor but probably is not part of the binding domain,[746] and the suggestion that enzymatic dephosphorylation may be essential for terminating the action of the kinase.[747] The whole process may be one of the earliest biochemical steps following insulin binding.[745]

Interactions of Proteins with Nucleic Acids. An example of mixed association of proteins arises in the formation of multi-enzyme complexes, an area under

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particularly active consideration in relation to eukaryotic aminoacyl-tRNA synthetases. It has been established that at least sixteen activities may be identified as part of a complex,[748] that hydrophobic interactions and possibly lipids are important in maintaining the integrity of the complex,[749] and that, at least as far as their kinetic behaviour is concerned, seven activities associated in a complex are functionally independent.[750]

In relation to protein–nucleic acid interactions, Vlassov and co-workers[751] have examined the interactions of two tRNAs with aminoacyl-tRNA synthetases and concluded that the specificity of the aminoacylation reaction is probably not related to the existence of a well defined recognition site. Schwarz and Watanabe developed a theory for analysing the thermodynamics and kinetics of the interactions of proteins with multiple sites on a nucleic acid[752] and applied it to the binding of a protamine to calf-thymus DNA,[753] showing that about twenty nucleotides are occupied by one protamine molecule. Low-angle *X*-ray analysis and electron microscopy of the complex formed between histone octamer and single-stranded DNA led to the conclusion that its morphology is very similar to that of the nucleosome. These and other results suggest that binding of the octamer to single- or double-stranded DNA requires little energy change and may be involved in the processes of replication and transcription of the 'active chromatin'.[754]

Specific Biological Systems. – *Blood-coagulation Proteins.* It is well established that a variety of macromolecular interactions play key roles in the blood-clotting process and in its regulation. Several of these interactions received detailed attention this year. Surface-pressure measurements were used to suggest that prothrombin and its amino-terminal sequence (fragment 1) adsorbed to phospholipid monolayers almost exclusively through phosphatidylserine head groups, and comparison of binding curves obtained with the monolayers and with bilayer vesicles revealed that curvature effects were unimportant in determining the binding response.[755] Thrombin formed by proteolysis of prothrombin on the surface of phospholipid vesicles interacts *inter alia* with platelets, endothelial cells, and fibrinogen, which is converted to fibrin. A study on the interaction of thrombin with fibrin led to the hypothesis that the binding to fibrin monomer and subsequent release favoured by Ca^{II}, or by indefinite self-association of fibrin to form the three-dimensional network, may be one determinant in controlling the effective thrombin concentration *in vivo*.[756]

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Another determinant is the inhibition of thrombin by complex formation with antithrombin, which was studied by a variety of techniques[757–759] with findings that thrombin is more susceptible to proteolytic attack when complexed with antithrombin[757] and that the primary action of heparin is to increase the affinity of the initial complex.[758] Heparin–antithrombin interactions were also studied by circular-dichroism spectroscopy,[760] and the regulation of thrombin action by small platelet release products received attention in relation to the linked binding sites on thrombin.[761] Indeed the range of interactions studied in this field continues to grow as exemplified by work on the interactions of bovine blood-clotting factor Va and its subunits with phospholipid vesicles,[762] of human-plasma kallikrein and its light chain with $C\bar{1}$ inhibitor,[763] and of high-molecular-weight forms of von Willebrand factor to fibrillar collagen, which facilitates subsequent platelet adhesion.[764]

In the specific area of fibrin association, we find a more refined delineation of the structure of human fibrinogen in terms of an antiparallel orientation of its two identical halves, each containing three peptide chains, α, β, and γ, linked by disulphide bonds.[765] Thrombin cleaves an A peptide from each of two Aα chains of fibrinogen, exposing a reactive site in the central domain of the molecule capable of interaction with a site already exposed in the terminal domain; the self-association that follows has been monitored by interrupting it at early stages using a thrombin inhibitor and by determining distributions of oligomer lengths by electron microscopy.[766] It appears from the latter work that thrombin releases the first A peptide more slowly than the second, with consequent time dependence of the valency of the mixture of associating monomer units ($A\alpha_2$ and α_2). Janmey and co-workers[767] have also commented on this model, utilizing light-scattering studies that provide a simultaneous measurement of the weight-average degree of association and the fraction of unmasked binding sites. We note also that monoclonal antibodies have been generated to α-chain regions of human fibrinogen that participate in polymer formation[768] and that inhibition of fibrin polymerization by fragment D is unlikely to have an anti-clotting effect under physiological conditions.[769] The basis of fibrinolysis, resulting in dissolution of fibrin clots, was probed further in two studies, one providing quantitative

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information on the binding parameters governing the interaction of plasminogen with fibrin and fibrinogen[770] and the other, relevant to the control of plasmin-catalysed fibrinolysis, discussing the crosslinking of α_2-plasmin inhibitor to the same acceptor.[771]

Actin Self-assembly. Considerable research activity has been directed towards the biphasic nature of the kinetics of filament formation from actin monomers. Some investigations have favoured the simplest polymerization-condensation mechanism in which the formation of an aggregate comprising four[772] or two to four[773] actin monomers precedes an elongation phase. However, other lines of evidence[774–777] indicate that the mechanism is more complex than nucleation followed by elongation. An additional activation step has been proposed, but there is some conflict as to whether it is the oligomeric nucleus[776] or the actin monomer[774] that undergoes activation; the monomer conformation under polymerizing conditions has been the subject of reinvestigation.[778, 779] A detailed mechanism consistent with monomer activation entails the interaction of Mg^{II} with a metal-binding site on G-actin monomer to induce a conformational change that is required for eventual polymerization.[777] In terms of this model, involving competition between Ca^{II} and Mg^{II} for the same G-actin site, the inhibitory effect of Ca^{II} on the kinetics of actin polymerization[772, 777, 780] reflects the need for Ca^{II} to be displaced by Mg^{II} for the conformational change to occur.[777] Even greater mechanistic complexity is indicated by the observation that different critical monomer concentrations (at which association and dissociation reactions are balanced) pertain at the two ends of actin filaments.[781] Studies of an actin dimer covalently crosslinked by *N,N'-p*-phenylenebisacrylamide have revealed the uncoupled nature of the processes of ATP hydrolysis and actin polymerization, and also the hydrolysis of filament-bound ATP in preference to dimer-bound ATP.[782]

Other investigations have been concerned with effects of various proteins on the polymerization of actin, the regulation by the 88 000 dalton protein–actin complex and cytochalasin B being one such example.[783] Calcium-activated factors

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[775] H. R. Gilbert and C. Frieden, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 404.
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[777] C. Frieden, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 6513.
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[779] J. A. Barden, C.-S. C. Wu, and C. G. Dos Remedios, *Biochim. Biophys. Acta*, 1983, **748**, 230.
[780] L. A. Selden, J. E. Estes, and L. S. Gerhman, *Biochem. Biophys. Res. Commun.*, 1983, **116**, 478.
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[782] S. C. Mockrin and E. D. Korn, *J. Biol. Chem.*, 1983, **258**, 3215.
[783] E. Nishida, Y. Ohta, and K. Sakai, *J. Biochem. (Tokyo)*, 1983, **94**, 1671.

that inhibit polymerization have been detected in the cytosol of pancreatic islets,[784] and two calcium-dependent modulation proteins have been isolated from bovine thyroid.[785] The larger protein ($M_r = 80\,000$) enhances nucleation and hence polymerization by binding to F-actin, whereas the smaller protein ($M_r = 40\,000$), an inhibitor of actin polymerization, binds to G-actin.[785] In similar vein, the inhibitory effect of vitamin D-binding protein is the consequence of its interaction with G-actin.[786] A survey has been made of calmodulin-binding proteins that interact with actin filaments in a calcium-dependent flip-flop manner,[787] and an analogy has been drawn between the effects of erythrocyte spectrin and bovine-brain calspectin, a calmodulin-binding protein that also crosslinks actin and thereby induces polymerization by enhancing nucleation.[788] Finally, the crosslinking of spectrin–actin complexes by band 4.1 protein results in a limited depolymerization of actin filaments, a phenomenon that may account in part for the existence of actin in the erythrocyte skeleton as short oligomers rather than as long filaments.[789]

Muscle-protein Interactions. The interaction between myosin (thick filament) and actin (thin filament) has continued to attract much attention. A quick-freeze, deep-etch replica technique of sample preparation has been used to visualize actin–myosin interactions, and it has yielded particularly distinct images of these crossbridges in insect flight muscle.[790, 791] Comparative studies of the binding of gizzard smooth-muscle myosin subfragment 1 and its skeletal muscle counterpart to actin in the presence and absence of ATP have led to the conclusion that the crossbridges in smooth muscle undergo a similar kinetic cycle to those in skeletal muscle but that they bind more tightly to the actin filaments.[792] Studies based on chymotryptic digestion and chemical cross-linking methods have shown that phosphorylation of the myosin light chains causes a partial release of crossbridges from the thick-filament surface[793] whereas Mg^{II} strengthens the association of crossbridges with the myosin-filament backbone.[794] In this regard, the fact that high-molecular-weight subfragment 2 of myosin undergoes self-association that is induced by the binding of divalent cations within the light-meromysin–heavy-meromyosin hinge region is also consistent with the concept that the binding of Mg^{II} may act to lock the cross-

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[790] J. E. Heuser and R. Cooke, *J. Mol. Biol.*, 1983, **169**, 97.
[791] J. E. Heuser, *J. Mol. Biol.*, 1983, **169**, 123.
[792] L. E. Greene, J. R. Sellers, E. Eisenberg, and R. S. Adelstein, *Biochemistry*, 1983, **22**, 530.
[793] A. Mrakovčić-Zenic and E. Reisler, *Biochemistry*, 1983, **22**, 525.
[794] E. Reisler, J. Liu, and P. Cheung, *Biochemistry*, 1983, **22**, 4954.

bridge on to the thick-filament surface.[795] Crosslinking of the two reactive sulphydryl groups of myosin subfragment 1 with *N,N'-p*-phenylenedimaleimide has produced a stable analogue of the subfragment 1–ATP complex,[796] which could well facilitate examination of actin–myosin crossbridges in the relaxed conformational state and hence comment upon a kinetic model proposed for the interaction of myosin subfragment 1 with regulated actin.[797] Three bis-(imidoester)s with 1.1 nm armlengths have been used to crosslink F-actin in an investigation designed to provide further evidence for the interaction of myosin-head heavy chain with an actin dimer.[798] The calcium-sensitive binding of heavy meromyosin to regulated actin requires the head–tail junction and the N-terminal portion of the 19 000 dalton light chains (LC2) to be intact, the calcium sensitivity being due to the troponin that is attached to the actin.[799] Other studies have considered the interaction of light chains with the heavy chain of myosin subfragment 1[800,801] and the location of the active site of subfragment 1 in relation to a particular cysteine residue (177) in the larger alkali light chain, LC1.[802]

Interactions occurring within the thin filament have not been examined so extensively in the year under review, but there are reports of electron-microscopy[803] and fluorescence-quenching[804] studies of the tropomyosin-troponin system. Of particular interest is the finding that troponin interacts with tropomyosin over a considerable proportion of its length, a factor that is possibly important in the dynamics of the calcium-sensitive switch that regulates muscle contraction.[803]

The binding of cytosolic enzymes to muscle structural proteins has also been examined. Even though creatine kinase is one of the M-line proteins detected by electron microscopy of muscle filaments, *in vitro* studies of mixtures of the enzyme and either myosin or myosin subfragments have failed to detect any interaction under conditions physiological with respect to pH and ionic strength, a result taken as signifying a requirement for additional components in order to simulate the situation pertaining in intact myofibrils.[805] In that regard, partition equilibrium studies of the binding of aldolase to myofibrils have certainly indicated a much stronger interaction than that detected with thin filaments alone.[806] On the basis of the partition equilibrium studies, plus the aldolase and thin-filament contents of muscle, resting muscle is indicated as containing a significant proportion (25–30%) of aldolase in the bound form, with changes in

[795] H. Ueno, M. E. Rodgers, and W. F. Harrington, *J. Mol. Biol.*, 1983, **168**, 207.
[796] J. M. Chalovich, L. E. Greene, and E. Eisenberg, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 4909.
[797] A. C. Balazs and I. R. Epstein, *Biophys. J.*, 1983, **44**, 145.
[798] J.-P. Labbé, D. Mornet, G. Roseau, and R. Kassab, *Biochemistry*, 1982, **21**, 6897.
[799] P. D. Wagner and D. B. Stone, *Biochemistry*, 1983, **22**, 1334.
[800] M. Burke, M. Sivaramakrishnan, and V. Kamalakannan, *Biochemistry*, 1983, **22**, 3046.
[801] P. D. Wagner and D. B. Stone, *J. Biol. Chem.*, 1983, **258**, 8876.
[802] D. J. Moss and D. R. Trentham, *Biochemistry*, 1983, **22**, 5261.
[803] P. F. Flicker, G. N. Phillips, jun., and C. Cohen, *J. Mol. Biol.*, 1983, **162**, 495.
[804] M. Lamkin, T. Tao, and S. S. Lehrer, *Biochemistry*, 1983, **22**, 3053.
[805] J. L. Woodhead and S. Lowey, *J. Mol. Biol.*, 1983, **168**, 831.

the subcellular distribution of the enzyme being likely to occur during exercise owing to the increased concentrations of Ca^{II} and fructose 1,6-bisphosphate that then prevail.[806]

Acknowledgement. – The authors are indebted to Elisabeth A. Owen for assistance with the literature search.

[806] M. R. Kuter, C. J. Masters, and D. J. Winzor, *Arch. Biochem. Biophys.*, 1983, **225**, 384.

3
Peptide Synthesis

BY I. J. GALPIN
Appendices compiled by
C. M. GALPIN

1 Introduction

In the last year there has been an increase in the number of published papers dealing with the synthesis of peptides and new methods for use in peptide synthesis. The solid-phase approach utilizing either polyamide or polystyrene supports now provides a viable route for the synthesis of most small peptides. Solution methods have also developed and still probably provide the best means of preparing large amounts of a given peptide sequence. In addition, solution methods are readily adaptable when modified peptides are to be synthesized.

Structure–activity relationships clearly play a major part in the design and synthesis of biologically active peptides, and to this end it is interesting to see the increasing role being played by computer graphics. There have been many examples of the study of substrate–receptor interactions, and a particularly elegant example is provided by work on the three-dimensional structure of renin and the binding site of angiotensinogen.[1] Such studies are clearly important in the development of renin inhibitors.

The Proceedings of the 25th Symposium on Peptide Chemistry at Toyonaka, Japan,[2] the Proceedings of the 17th European Peptide Symposium in Prague,[3] and the Proceedings of an International Symposium on Peptides and Proteins at Galzignano, Italy,[4] have been published. No attempt has been made to cover the content of these meetings in detail, and in many cases it is clear that material that has not been published elsewhere has been presented. The first two references[2,3] deal mainly with synthesis, whereas in the last reference[4] more biological aspects are covered.

Other interesting texts which have been noted this year include 'Peptides; Synthesis and Physical Data';[5] this work in six volumes presents the synthesis of

[1] T. Blundell, B. L. Sibanda, and L. Pearl, *Nature (London)*, 1983, **304**, 273.
[2] 'Peptide Chemistry, 1982', Proceedings of the 20th Symposium on Peptide Chemistry, Protein Research Foundation, Toyonaka, Japan, 1983.
[3] 'Peptides 1982', Proceedings of the 17th European Peptide Symposium, Prague, ed. K. Blaha and P. Malon, Walder de Gruyter, Berlin, New York, 1983.
[4] Proceedings of an International Symposium, Galzignano, Italy, ed. M. Goodman, *Biopolymers*, 1983, **22** (1).
[5] 'Peptides: Synthesis, Physical Data', W. Voetler and E. Schmid-Siegmann, Georg Thieme Verlag, Stuttgart, New York, 1983.

three thousand or so amino acid derivatives and small peptides in a computerized format, which is intended to provide ready reference for laboratory workers. Also, Volume 5 of 'The Peptides, Analysis, Synthesis, Biology'[6] has appeared. This volume deals with special methods in peptide synthesis and covers side reactions in peptide synthesis, racemization, dehydroamino acids and peptides, unusual amino acids, and the synthesis of peptides by recombinant DNA methods.

The lay-out of this chapter follows that established previously, with the Appendices providing coverage of virtually all the published literature in the area.

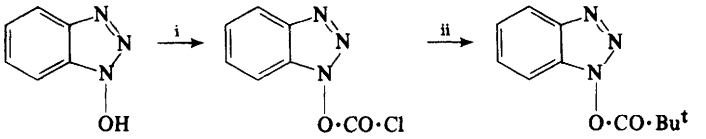
2 Methods

Protective Groups. – *Established Methods of Amino-group Protection.* The use of di-t-butyl dicarbonate for the introduction of the t-butyloxycarbonyl (Boc) protecting group has been investigated by means of accurate pH control employing a pH stat.[7] A new reagent, t-butylamino carbonate (1), has been prepared by reaction of hydroxylamine with di-t-butyl dicarbonate.[8] The reaction, which was carried out in 50% dioxan at 0 °C and pH 10.3 in the presence of 4M sodium hydroxide, gave the amino carbonate (1) when di-t-butyl dicarbonate was used in excess. However, if excess hydroxylamine was used then the isomeric compound (2) was produced. Compound (1) may also be converted to (2) in the presence of 5% hydroxylamine in 50% dioxan at pH 9.3. Compound (1) was claimed to be an excellent acylating agent, reacting at between 1.5 and 2.5 times the rate of the corresponding symmetrical carbonate, and amino acids were readily acylated in acidic solution at pH 5.5. It is interesting to note that compound (1) is a potent acylating agent whereas compound (2) is not.

$$\underset{(1)}{Bu^tO{\cdot}CO{\cdot}O{\cdot}NH_2} \qquad \underset{(2)}{Bu^t{\cdot}O{\cdot}CO{\cdot}NH{\cdot}OH}$$

t-Butyl benzotriazolylcarbonate (3) has been prepared from HOBt by the route outlined in Scheme 1.[9] The so-called BDC reagent (3) has been used to prepare Boc-amino acids. The reagent and amino acid are stirred together in aqueous dioxan in the presence of triethylamine for 10 minutes at room temperature, giving a yield in the 80–90% region. The reagent can be used to advantage with DMF as solvent for the acylation of relatively unreactive amines.

[6] 'The Peptides, Analysis, Synthesis, Biology', ed. E. Gross and J. Meienhofer, Academic Press, New York, 1983.
[7] G. Perseo, S. Piani, and R. de Castiglione, *Int. J. Pept. Protein Res.*, 1983, **21**, 227.
[8] R. B. Harris and I. B. Wilson, *Tetrahedron Lett.*, 1983, **24**, 231.
[9] S. Kim and H. Chang, *J. Chem. Soc., Chem. Commun.*, 1983, 1357.
[10] G. F. Sigler, W. D. Fuller, N. C. Chaturvedi, M. Goodman, and M. Verlander, *Biopolymers*, 1983, **22**, 2157.
[11] M. Tessier, F. Albericio, E. Pedroso, A. Grandas, R. Eritja, E. Giralt, C. Granier, and J. Van Rietschoten, *Int. J. Pept. Protein Res.*, 1983, **22**, 125.



(3)

Reagents: i, $COCl_2$/pyridine; ii, Bu^tOH/pyridine

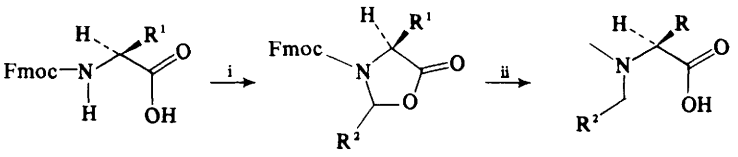
Scheme 1

The 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group is now used widely in solid-phase synthesis. Several workers have encountered difficulty in the preparation of Fmoc-amino acids, and the presence of Fmoc-dipeptides as contaminants in Fmoc-amino acids has been noted.[10,11] It appears that a mixed anhydride formed from the Fmoc-amino acid and Fmoc chloride is implicated in the production of the unwanted Fmoc-dipeptide. Amino acid residues with small aliphatic side chains seem particularly prone to this side reaction, and in the case of Fmoc-glycine the simple procedure can lead to the production of between 10 and 20% of Fmoc-glycyl glycine. The presence of Fmoc-Gly_3-OH (0.02%) was also observed.[10] As a solution to this problem the use of 20–25% excess Fmoc chloride over the amino acid is suggested;[10] using a minimum volume of dioxan to aqueous phase, four equivalents of sodium carbonate were used, and the reaction mixture was stirred vigorously. An alternative solution[11] is to convert the Fmoc chloride to the corresponding azide, using sodium azide, as this seems to give a cleaner reaction. It is important to note that Fmoc-dipeptide contamination is often particularly difficult to remove and is not removed easily by repeated crystallization. Fmoc-ONSu and Troc-ONSu have also been prepared,[12] and the former has been used for the introduction of Fmoc protection under Schotton–Baumann conditions. Using this reagent, Fmoc-dipeptides were not found as contaminants in the Fmoc-amino acids produced by this route. The Troc-ONSu was used similarly for the introduction of the trichloroethoxycarbonyl protecting group.

Synthesis of Fmoc-*N*-methyl- or -*N*-alkyl-amino acids by reduction of an oxazolidinone has been reported.[13] In this method the Fmoc-amino acid is converted to the oxazolidinone (4) by the route shown in Scheme 2. Reduction of the oxazolidinone then yields the Fmoc-*N*-alkylamino acid. No racemization was encountered during this synthesis as demonstrated by the use of the chiral shift reagent tris(heptafluorobutyl)camphoratoeuropium(III).

Frequently *N*-formyl peptides are required for physiological work, and to this end the reagent trimethylacetic formic mixed anhydride has been prepared.[14] The reagent formed by reaction of sodium formate with pivaloyl chloride in the presence of 18-crown-6 is a highly efficient and selective formylating agent.

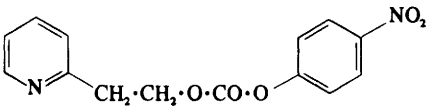
[12] L. Lapatsanis, G. Milias, K. Froussions, and M. Kolovos, *Synthesis*, 1983, 671.
[13] R. M. Freidinger, J. S. Hinkle, D. Perlow, and B. H. Arison, *J. Org. Chem.*, 1983, **48**, 77.
[14] E. J. Vlietstra, J. W. Zwikker, R. J. M. Nolte, and W. Drenth, *Recl. Trav. Chim. Pays-Bas*, 1982, **101**, 460.



(4)

Reagents: i, R^2CHO/TosOH/toluene/Δ; ii, (*a*) Et_3SiH, (*b*) TFA/$CHCl_3$ (1/1)

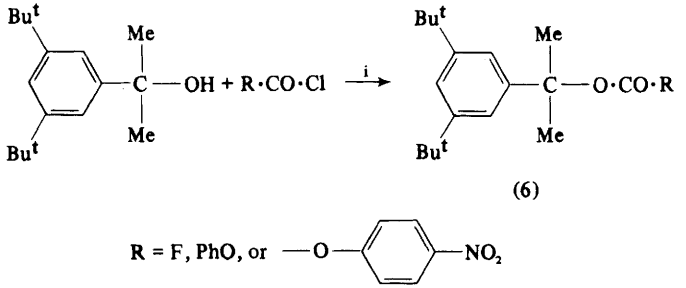
Scheme 2



(5)

New Methods of Amino-group Protection. The 2-(2-pyridyl)ethoxycarbonyl- (Pyoc) protecting group has been evaluated for use in peptide synthesis.[15] The protecting group is introduced by means of the mixed carbonate (5) by reaction with the free amino acid at pH 9.5 in acetonitrile water (1:1); yields of 85–100% are reported. The group, which is stable to both acid and base, promotes water solubility, and thus coupling may be carried out using water-soluble carbodiimides and HOBt; alternatively a traditional non-aqueous approach may be used, with mixed anhydrides or DCCI in the presence of HONSu. Removal of the protecting group is effected by treatment with 20% dimethylamine in DMF, the free amine being liberated by elimination by an E1cB mechanism. The protecting group would not, of course, be applicable if cysteine or methionine were present, as side reactions could be anticipated on reaction with methyl iodide.

The 1-(3,5-di-t-butylphenyl)-1-methylethoxycarbonyl (t-Bumeoc) group has been developed as a new, very acid, labile amino-protecting group.[16] The protecting group is introduced by the use of an activated intermediate of the type (6) shown in Scheme 3. Reaction of one of the activated intermediates of type (6) with an amino acid rapidly gives the t-Bumeoc-protected amino acid in high



(6)

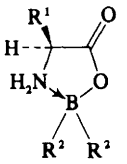
Reagent: i, pyridine

Scheme 3

[15] H. Kunz and R. Barthels, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 783.

yield. The utility of the protecting group was demonstrated by the synthesis of HPhe-D-Leu-Arg-Phe-NH_2, in which the protecting group was cleaved by treatment with 3% TFA in dichloromethane. It may also be cleaved by treatment with mixtures of acetic acid:formic acid:water (7:1:2) or 80% acetic acid; it is stable to hydrogenolysis and to alkali. In a study of acid lability it was shown that the group was extremely labile, and in comparison with other groups it showed the following order of cleavage: Boc < Adpoc < Ppoc < Bpoc < t-Bumeoc (rates of cleavage 1:600:750:2000:8000).

Boroxazolidones have been investigated for the simultaneous protection of amino and carboxyl groups of α-amino acids.[17] These derivatives are prepared by reaction of the free amino acid with triethyl boron or triphenyl boron in THF. Formation of the derivative (7) involves complexation of both the carboxyl and



(7) R^2 = Et or Ph

amino functions of the α-amino acid, thus allowing manipulation of functional groups present in the side chain R^1. This mode of co-ordination was clearly demonstrated by the preparation of β-aspartyl and γ-glutamyl benzyl esters. In both cases the complex (7) was reacted with dicyclohexylamine and benzyl bromide in DMF; both derivatives gave the side-chain substituted ester. Lysine could also be side-chain formylated using these derivatives by reaction with trimethylacetic formic anhydride mentioned above.[14] The derivatives are generally cleaved by solvolysis with HCl in methanol, and it appears that the derivative with R^2 = ethyl is slightly superior to that with R^2 = phenyl as the latter compound is rather stable towards solvolysis.

The 1-benzotriazolylcarbonyl derivatives mentioned in Volume 15 of this Report (page 319) have been used in the synthesis of di- and tri-peptides.[18] The protecting group in this case acts as an amino-protecting group and provides carboxyl activation.

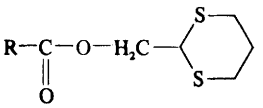
Carboxyl Protection. 9-Fluorenylmethyl esters have been investigated by two research groups.[19,20] These esters may be prepared by coupling of 9-hydroxymethylfluorene with an *N*-protected amino acid in the presence of DCCI and dimethylamino pyridine[19] or by imidazole-catalysed transesterification of active esters.[20] The 9-fluorenylmethyl esters are stable to transesterification on heating in alcohol and may be removed by β-elimination on treatment with base.

[16] W. Voelter and J. Mueller, *Liebigs Ann. Chem.*, 1983, 248.
[17] G. H. L. Nefkens and B. Zwanenburg, *Tetrahedron*, 1983, **39**, 2995.
[18] I. Butula, B. Zorc, and M. Ljubic, *Synthesis*, 1983, 327.
[19] H. Kessler and R. Siegmeier, *Tetrahedron Lett.*, 1983, **24**, 281.
[20] M. A. Bednarek and M. Bodanszky, *Int. J. Pept. Protein Res.*, 1983, **21**, 196.

Cleavage conditions are similar to those employed for the Fmoc-amino-protecting group, that is using secondary amines, *e.g.* dimethylamine, morpholine, or piperidine. Cleavage is also effected by treatment with triethylamine or indeed by prolonged treatment with amino acid esters or amides.[20] It is interesting to note that such cleavage may be suppressed by the addition of HOBt. Although benzyloxycarbonyl protection could be removed by treatment with HBr in acetic acid or trifluoroacetic acid in the presence of thioanisol, selective cleavage of the Z group could not be achieved by hydrogenolysis as under the standard conditions of benzyloxycarbonyl removal the 9-fluorenylmethyl ester is slowly cleaved.

Carboxamidomethyl esters (CAM esters) have been prepared by reaction of *N*-protected amino acid caesium salts with α-chloroacetamide.[21] The esters are stable to TFA and to hydrogenolysis and are not cleaved by organic bases such as cyclohexylamine or triethylamine in DMF. Cleavage may be effected by treatment with excess sodium hydroxide (0.5M) in between 10 minutes and 2 hours, or by treatment with sodium carbonate in DMF–water. Sodium bicarbonate may also be used, but under these conditions the hydrolysis time is considerably extended. It is claimed that no racemization is encountered during the formation or cleavage of these esters.

1,3-Dithian-2-ylmethyl esters (Dim esters) have been prepared by transesterification of amino acid methyl esters with dithianylmethyl alcohol using aluminium isopropoxide as a catalyst.[22] Such esters cannot be prepared by acid-catalysed methods owing to the instability of the dithianylmethyl alcohol. The esters of general structure (8) are stable to acid and base and are removed by oxidation to the corresponding bis-sulphone by hydrogen peroxide in the presence of ammonium molybdate, deprotection being achieved by an E1cB mechanism at pH 8. Clearly, these esters cannot be used if the peptide contains amino acids that are sensitive to oxidation.



(8)

The advantages of using the picolyl ester method have been demonstrated in a synthesis of prothrombin (202–215).[23] In this synthesis efficient purification of intermediates was carried out by using either work-up with aqueous citric acid or ion-exchange chromatography on Amberlyst 15. Ultimately the picolyl ester was cleaved by treatment with zinc in acetic acid.

A convenient preparation of t-butyl esters using DMF dibutylacetal has been reported.[24] In this method the *N*-protected amino acid is reacted with the acetal

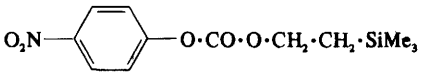
[21] J. Martinez, J. Laur, and B. Castro, *Tetrahedron Lett.*, 1983, **24**, 5219.
[22] H. Kunz and H. Waldmann, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 62.
[23] A. Hallett, A. P. Hope, M. S. Munns, R. Richardson, and G. T. Young, *J. Chem. Res. (S)*, 1983, 32.
[24] U. Widmer, *Synthesis*, 1983, 135.

at 80 °C, giving a good yield of the corresponding t-butyl ester. Preparation of ethyl or benzyl esters of amino acids, particularly the acid-sensitive tryptophan residue, by refluxing the amino acid with the alcohol in the presence of tosyl chloride has also appeared.[25]

A simple procedure for the esterification of *N*-protected amino acids has been reported;[26] by this method the *N*-protected amino acid is reacted with trimethylsilyl chloride in dry THF at reflux. The resulting trimethylsilyl ester is then treated with the appropriate alcohol to bring about conversion to the corresponding ester.

Side-chain Protection. The preparation of Troc-glutamyl- and Troc-aspartyl-α-ethyl and -α-benzyl monoesters has been reported.[27] Preparation of these compounds was achieved by opening the Troc-glutamic acid or -aspartic acid anhydrides with ethyl or benzyl alcohol. Fractional crystallization finally gave the pure α-ester. *N*-Trityl-*O*-alkylhydroxyamino acids have been prepared and used in synthesis.[28] The tritylhydroxyamino acid was treated with sodium hydride and imidazole and then alkylated with an alkyl halide. The authors claim that the particularly easy detritylation of such compounds provides an efficient route to the *O*-alkylamino acid.

The preparation of side-chain-protected trimethylsilylethoxycarbonyl derivatives of lysine, ornithine, and diaminobutyric acid using the mixed carbonate (9) has been carried out.[29] The preparation of these derivatives, which was carried out by reaction of the mixed carbonate (9) with the copper complex of these diamino acids, gave a 90–95% yield of the side-chain-substituted product on treatment with EDTA. There was a slight suggestion that the side-chain Teoc



(9)

group was labile to acid, but Boc could be removed selectively in the presence of the Teoc group by the use of tosic acid (60–65 °C for 20 min). The group is also known to be stable to hydrogenolysis. Thus, use of side-chain Teoc protection in combination with main-chain Z or Boc protection is feasible.

The side-chain protection of arginine during solid-phase peptide synthesis has been considered in detail.[30] Several side-chain derivatives of Fmoc arginine have been considered, and both methoxybenzene sulphonyl and bis-adamantyloxycarbonyl arginine were found to be unsatisfactory as in the first case methane

[25] I. Aari and I. Muramatsu, *J. Org. Chem.*, 1983, **48**, 121.
[26] M. A. Brook and T. H. Chan, *Synthesis*, 1983, 201.
[27] J. F. Carson, *Synthesis*, 1983, 669.
[28] K. Barlos, D. Papaioannou, P. Cordopatis, and D. Theodoropoulos, *Tetrahedron*, 1983, **39**, 475.
[29] A. Rosowsky and J. E. Wright, *J. Org. Chem.*, 1983, **48**, 1539.
[30] E. Atherton, R. C. Sheppard, and J. D. Wade, *J. Chem. Soc., Chem. Commun.*, 1983, 1060.

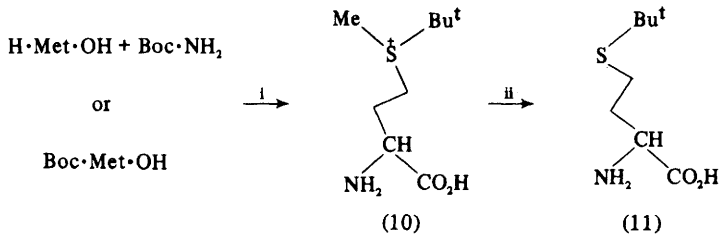
sulphonic acid was required for deprotection and the second derivative was too lyphophilic and was extremely difficult to prepare. The 4-methoxy-2,3,6-trimethylbenzene sulphonyl group (Mtr) was found to be the most stable and was readily cleaved by treatment with TFA in thioanisole in one hour. The Mtr group is completely stable under the conditions required for Fmoc removal, and as stated above may be removed with TFA in the presence of thioanisole. However, in the presence of scavengers such as ethane dithiol deprotection of the Mtr group may take up to five hours. Fmoc-Arg(Mtr)-OH was found to be slow to couple, but when incorporated gave satisfactory results in the synthesis of *cro* repressor protein (4–15). It should be noted that the cleavage using TFA–thioanisole gave a purer product than when TFA–thiophenol[31] was used.

The tryptophan indole nitrogen is now frequently protected, especially in solid-phase peptide synthesis. N^{in}-Formyl tryptophan was used to particular advantage in the synthesis of opioid peptide E.[32] In the synthesis of this 25-residue peptide, using a Pam resin, the tryptophans at 13 and 14 were both ultimately protected with a formyl group as the product was much more readily purified than when the indole nitrogen was unprotected. The 2,2,2-trichloroethoxycarbonyl (Troc) protecting group has also been investigated, as the protecting group for the indole nitrogen.[33] The protecting group was introduced by reaction of Boc-Trp-OBzl with Troc chloride in the presence of sodium hydroxide and tetrabutylammonium hydrogen sulphate as a catalyst. Hydrogenolysis using 5% palladium on charcoal removed the benzyl group cleanly, but it should be noted that prolonged hydrogenolysis gives rise to some impurities. The N^{in}-Troc group was stable to trifluoromethane sulphonic acid–trifluoroacetic acid–*o*-cresol over four hours at room temperature, but it was removed by treatment with 0.01M sodium hydroxide in methanol over two hours. Alternative deprotection may be achieved using hydrazine in methanol, or cadmium dust in a mixture of acetic acid and DMF. It is worth noting that incomplete deprotection of the Troc function was observed when zinc was used for deprotection.

The protection of the thiol function of cysteine by the dimethylphosphinothionyl (Mpt) group has been studied.[34] The group is introduced by reaction of Mpt chloride with cysteine under Schotton–Baumann conditions. Under suitable conditions Mpt-Cys(Mpt)-OH is isolated as its DCHA salt. *S*-Mpt is stable to acid, with the exception of HBr in acetic acid, and is removed by silver nitrate or mercuric acetate. *N*-Mpt may be removed selectively in the presence of *S*-Mpt by alkaline hydrolysis, although under these conditions some β-elimination to give dehydroalanine is observed.

Transformation of methionine into *S*-t-butyl homocysteine has been evaluated as a method of converting methionyl peptides to homocysteinyl peptides.[35] The transformation has been applied to methionine itself and to the conversion of substance P to t-butylhomocysteinyl[11] substance P. The general method is that

[31] M. Fujino, M. Wakimasu, and E. Kitada, *Chem. Pharm. Bull. Jpn.*, 1981, **29**, 2825.
[32] E. P. Heimer, T. J. Lambros, A. M. Felix, G. Fleminger, C. H. Li, M. Westphal, and J. Meienhofer, *Arch. Biochem. Biophys.*, 1983, **225**, 518.
[33] Y. Kiso, M. Inai, K. Kitagawa, and T. Akita, *Chem. Lett.*, 1983, 739.
[34] M. Ueki and K. Shinozaki, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1187.
[35] G. Chassaing, S. Lavielle, and A. Marquet, *J. Org. Chem.*, 1983, **48**, 1757.



Reagents: i, HF; ii, $HO{\cdot}CH_2CH_2{\cdot}S^-Na^+$/DMF

Scheme 4

indicated in Scheme 4. The reaction involves the generation of the intermediate sulphonium salt (10), which is then demethylated to give *S*-t-butyl homocysteine (11). The reaction has been carried out in the presence of free tryptophan without damage to this residue occurring. The *S*-t-butyl function may be removed from t-butyl homcysteine derivatives by reaction of Nps·Cl, mercuric acetate at pH 4, or mercuric trifluoroacetate. It is also removed by treatment with HF at 0 °C for 10 minutes.

General Deprotection. – Total deprotection is still frequently carried out using HF, and in a recent synthesis of human parathyroid hormone (1–84) an efficient deprotection was achieved using HF in the presence of anisole, methionine, dimethylsulphide, and ethane dithiol for one hour at 0 °C.[36] Although this final deprotection was highly satisfactory, many other workers have been concerned over HF deprotection.

The mechanism of HF deprotection in the presence of scavengers has been investigated in detail.[37,38] Deprotection of, for example, *O*-benzyl serine occurs by an S_N2 mechanism when the weak base dimethyl sulphide is used as an HF diluent. However, when the concentration of HF in DMS falls below 55% little unwanted alkylation occurs as carbonium ions are not formed. However, when the HF concentration increases above 55% the alkylation side reaction with, for example, tyrosine increases. HF in DMS will also reduce methionine sulphoxide to methionine, and, in the presence of a thiol, formyl tryptophan is also deprotected. When low concentrations of HF in DMS were used to deprotect benzyl esters of aspartic acid and glutamic acid the acylation side reactions were also minimized. It is suggested[37] that a mixture of HF–DMS–*p*-cresol (25:65:10 by volume) should generally be used and, when *N*-formyl tryptophan is also involved, that HF–DMS–*p*-cresol–*p*-thiocresol (25:65:7.5:2.5) should be used. In some cases it was found to be beneficial to use a two-stage deprotection procedure (the so-called 'low–high' procedure) whereby initially an S_N2 deprotection was carried out using a low HF concentration, and then finally, having

[36] T. Kimura, M. Takai, K. Yoshizawa, and S. Sakakibara, *Biochem. Biophys. Res. Commun.*, 1983, **114**, 493.
[37] J. P. Tam, W. F. Heath, and R. B. Merrifield, *J. Am. Chem. Soc.*, 1983, **105**, 6442.
[38] J. P. Tam, W. F. Heath, and R. B. Merrifield, *Int. J. Pept. Protein Res.*, 1983, **21**, 57.

removed groups which could give rise to carbonium ions, a high HF concentration was employed in order to sever the resin linkage.

Alkylation of tyrosine during HF deprotection was examined in detail,[38] and in this work it became clear that the effectiveness of the scavenger may readily be predicted by consideration of its pK_a. The severity of alkylation of tyrosine followed the order t-butyl < cyclohexyl < 2,6-dichlorobenzyl < 2-chlorobenzyl < benzyl. It is believed that the t-butyl carbonium ion has more time to diffuse through the solvent cage, and thus be trapped, giving rise to less alkylation. Clearly, from this work[37,38] it is important to establish optimal conditions for final deprotection, and it appears that the precise choice and concentration of scavenger are critical.

Frequently sulphonic acids have been used to replace HF in deprotection, and in a synthesis of metallothionein(II) (36-61) it was demonstrated that methane sulphonic acid was superior to HF for total deprotection.[39] Several other examples of the use of either trifluoromethane sulphonic acid or methane sulphonic acid in the presence of thioanisol and/or metacresol have been published,[40-42] and frequently trifluoroacetic acid has been added to this medium.[43] In a recent synthesis of rimorphin[44] a trifluoroacetic acid–thioanisole deprotection system was used for final deprotection.

Deprotection using sodium in liquid-ammonia reduction has again been re-examined,[45] and it was found that the definite blue colour associated with the normal end point of the removal of side-chain protection was unnecessary. In fact, side reactions could be suppressed or eliminated by optimizing the sodium consumption. If the sodium consumption was not measured accurately then several side reactions, including cleavage of Boc groups, $N-C_\alpha$ bond cleavage, reduction of side-chain amides to carbinols, transpeptidation, and hydantoin formation, can occur.

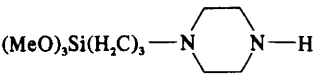
Catalytic-transfer hydrogenation has again been used,[46-48] and it is interesting to note that benzyloxycarbonyl can be removed by catalytic-transfer hydrogenation from methionyl peptides. The method has also been used with ammonium formate as donor for the release of synthetic peptides from Merrifield resins during solid-phase synthesis. The reaction is claimed to be virtually quantitative and compares favourably with the results obtained using HF deprotection. If the

[39] N. Ohta, Y. Okada, and K. Tanaka, *Chem. Pharm. Bull.*, 1983, **31**, 1885.
[40] K. Kitagawa, N. Kawai, Y. Kiso, T. Akita, K. Fukui, H. Amano, and H. Takagi, *Chem. Pharm. Bull.*, 1983, **31**, 2349.
[41] N. Fujii, *Yakugaku Zasshi*, 1983, **103**, 805.
[42] H. Yajima, N. Fujii, M. Shimokura, K. Akaji, S. Kiyama, and M. Nomizu, *Chem. Pharm. Bull.*, 1983, **31**, 1800.
[43] N. Fujii, W. Lee, H. Yajima, M. Moriga, and K. Mizuta, *Chem. Pharm. Bull.*, 1983, **31**, 3503.
[44] Y. Kiso, M. Inai, K. Kitagawa, T. Akita, and H. Moritoki, *Chem. Pharm. Bull.*, 1983, **31**, 1818.
[45] I. Schoen, T. Szirtes, T. Ueberhardt, A. Rill, A Csehi, and B. Hegedues, *Int. J. Pept. Protein Res.*, 1983, **22**, 92.
[46] K. M. Sivanandaiah and S. Gurusiddappa, *Indian J. Chem., Sect. B*, 1982, **21**, 857.
[47] M. K. Anwer, A. F. Spatola, C. D. Bossinger, E. Flanigan, R. C. Liu, D. B. Olsen, and D. Stevenson, *J. Org. Chem.*, 1983, **48**, 3503.
[48] A. Ljungqvist and K. Folkers, *Z. Naturforsch., Teil B*, 1983, **38**, 1022.

hydrogenation is carried out under neutral conditions, benzyl ethers of serine are cleaved with difficulty; however, with added acetic acid the cleavage proceeds smoothly.[47] In a solid-phase synthesis of some leukocyte interferon fragments it was found that hydrogenolysis using palladium generated *in situ* as catalyst was superior to the use of transfer hydrogenation using ammonium formate or cyclohexene as donor.[48] Catalytic activity may also be moderated using palladium in different oxidation states.[49] Whereas the palladium(II)-phthalocyanine complex is able to cleave benzyl esters leaving trichloro-t-butyloxycarbonyl groups intact, the similar complex palladium(I)-phthalocyanine cleaves the TcBoc group leaving the benzyl ester intact.

As mentioned above, the removal of the Troc group using zinc may give rise to unwanted side reactions. However, if cadmium replaces zinc then the deprotection proceeds smoothly.[50] Also, the Troc group was found to be unstable to hydrogenolysis over palladium, with some replacement of chlorine being observed.

During synthesis employing Fmoc for amino protection it has been noted that some problems may be encountered due to the addition of the amino component to the fulvene that is liberated on Fmoc cleavage. In order to combat this problem, piperazine or piperidine has been linked to a support so that the unwanted fulvene can be trapped during deprotection.[51,52] Polystyrene-based scavenging agents were found to be unsatisfactory, as frequently tedious washing procedures were required. However, using the silane (12), piperazine can be bound to an activated silica support. The supported organic base can then be used to scavenge dibenzofulvene; the results indicated that the piperidino system was superior to the piperazino-bound reagent, but in no case was complete scavenging achieved.



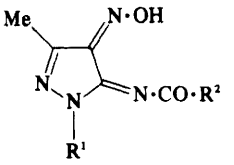
(12)

A new method of deblocking *O*-nitrophenylsulphenyl-protected peptides using ammonium thiocyanate and 2-methyl-1-indolylacetic acid has been reported.[53] The indolylacetic acid acts as a scavenger as *O*-Nps thiocyanate is formed reversibly and reacts with 2-methyl-1-indolylacetic acid to give 2-methyl-3-(2-nitrophenylthio)-1-indolylacetic acid, which can readily be extracted from organic media with dilute potassium carbonate. The method gave relatively slow cleavage with larger peptides.

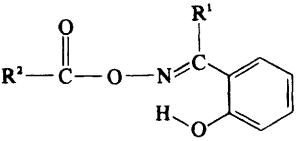
[49] H. Eckert, G. Fabry, Y. Kiesel, G. Raudaschl, and C. Seidel, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 881.
[50] I. J. Galpin, G. Hancock, G. W. Kenner, and B. A. Morgan, *Tetrahedron*, 1983, **39**, 149.
[51] L. A. Carpino, E. M. E. Mansour, C. H. Cheng, J. R. Williams, R. MacDonald, J. Knapczyk, M. Carman, and A. Lopusinski, *J. Org. Chem.*, 1983, **48**, 661.
[52] L. A. Carpino, E. M. E. Mansour, and J. Knapczyk, *J. Org. Chem.*, 1983, **48**, 666.
[53] I. F. Luescher and C. H. Schneider, *Helv. Chim. Acta*, 1983, **66**, 602.

Deblocking of ester functions has also received some attention. It was claimed that high yields of carboxylic acids may be obtained from benzhydryl esters by using formic acid for deprotection[54] and that care should be taken when hydrolysing peptides related to methionine-enkephalin with base, as alkaline saponification can lead to urea formation even when equivalent molar quantities of sodium hydroxide are used.[55]

Formation of Peptide Bonds. – The active-ester method continues to be a popular method of peptide-bond formation. A wide range of Fmoc-amino acid pentafluorophenyl active esters has been prepared by reaction of the Fmoc-amino acid with pentafluorophenol in the presence of DCCI.[56] A new type of active ester involving pyrazolines of the type (13) has been reported.[57] It is claimed that, using this reagent and also DCCI in the presence of HOBt, active esters may be prepared and that enhanced rates of reaction and yields are achieved with the minimum number of protecting groups.



(13) R^1 = Me or Ph
R^2 = EtO^- or $Z{\cdot}NH{\cdot}CH_2^-$



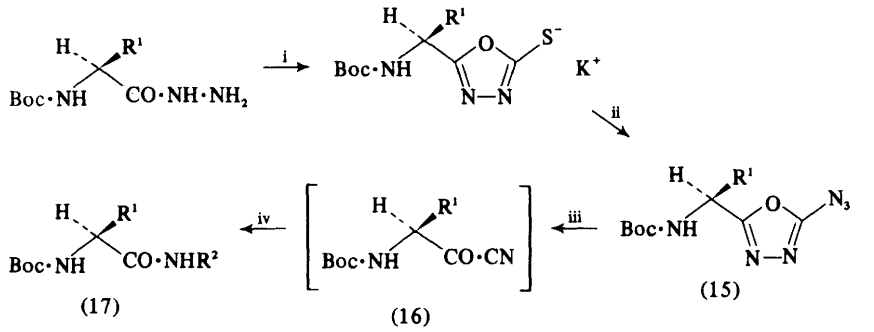
(14) R^1 = H or Me

The reactivity of aromatic *o*-hydroxyoxime amino acid active esters of general formula (14) has been investigated.[58] These active esters are best prepared by a reaction of the corresponding oxime with DCCI; the phenolic hydroxyl group was left unprotected as it provided intramolecular acid catalysis. If this acidic proton is protected, or the phenolic hydroxyl group is absent, additional weak-acid catalysis is required to enhance reactivity.

An elaborate synthesis of peptides based on the photochemistry of 5-azido-1,3,4-oxadiazoles has been proposed.[59] Preparation of the 5-azido-1,3,4-oxadiazole (15) follows the route outlined in Scheme 5. Photolysis at 375 nm leads to the acyl cyanide (16), which in turn is aminolysed to give the product (17). The method is complex, and the possibility of racemization *via* the acyl cyanide was not considered.

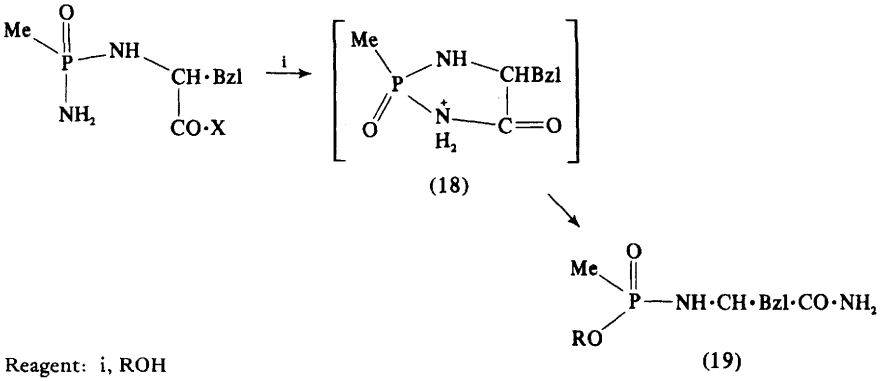
Amino-group transfer in cyclic phosphonic carboxylic imides has been demonstrated (see Scheme 6).[60] The cyclic intermediate (18) generated on

[54] T. Kametani, H. Sekine, and T. Honda, *Chem. Pharm. Bull.*, 1982, **30**, 4545.
[55] W. Voelter and A. Altenburg, *Liebigs Ann. Chem.*, 1983, 1641.
[56] L. Kisfaludy and L. Schon, *Synthesis*, 1983, 325.
[57] P. Giori, A. C. Veronese, C. B. Vincentini, G. Montanari, M. Guarneri, and C. A. Benassi, *Eur. J. Med. Chem.*, 1982, **17**, 515.
[58] I. Hayashi and K. Shimizu, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 3197.
[59] P. N. Confalone and R. B. Woodward, *J. Am. Chem. Soc.*, 1983, **105**, 902.
[60] N. E. Jacobsen and P. A. Bartlett, *J. Am. Chem. Soc.*, 1983, **105**, 1613.



Reagents: i, CS_2, KOH/MeOH; ii, (*a*) MeI, $KMnO_4$/HOAc, (*b*) NaN_3/EtOH; iii, *hν*/375 nm, benzene, 25 °C; iv, R^2NH_2

Scheme 5



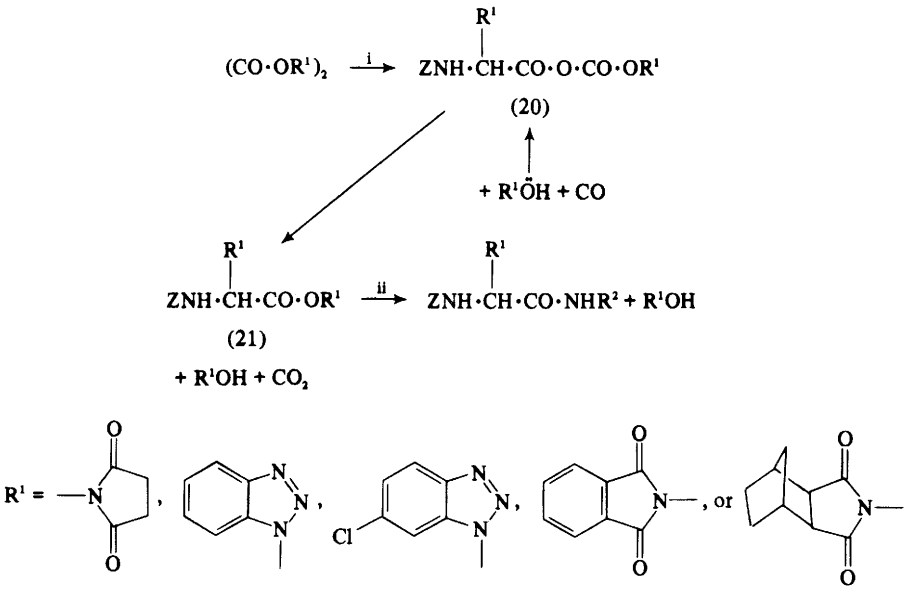
Reagent: i, ROH

Scheme 6

solvolysis leads to the production of (19). At present it is not clear whether this method of amide formation could be extended to the formation of peptide bonds. A convenient synthesis of peptides using oxalates has been reported.[61] The oxalate active esters (20) (see Scheme 7), which are prepared by a reaction of oxalyl chloride with two equivalents of the *N*-hydroxy compound, are reacted with an *N*-protected amino acid to give the mixed anhydride of type (20). This then reacts with 1 mole of liberated *N*-hydroxy compound to give the active ester of type (21), which in turn reacts with the amino component to give the product. It is claimed that the reagent is more convenient to use than DCCI and that a wide range of dipeptides has been prepared.

The use of mixed anhydrides of carboxylic or carbonic acids in peptide synthesis frequently suffers from a side reaction in which urethane formation may occur. The undesired opening of the non-symmetrical mixed anhydride

[61] K. Takeda, I. Sawada, A. Suzuki, and H. Ogura, *Tetrahedron Lett.*, 1983, **24**, 4451.



Reagents: i, ZNH·CHR¹·CO_2H/pyridine; ii, R^2NH_2

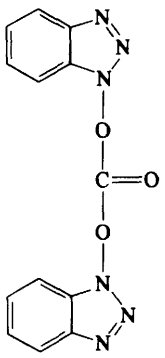
Scheme 7

has been found to be strongly influenced by the nature of the base used.[62] In THF, triethylamine gave more urethane than *N*-methylmorpholine, whereas in DMF the difference between these two bases was marginal. Also, in dichloromethane, triethylamine gave more urethane, particularly for hindered residues. *N*-methylmorpholine in dichloromethane, however, gave less urethane.

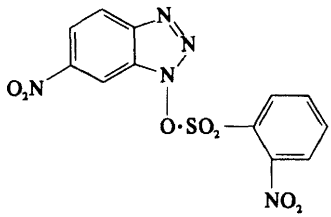
The use of *N*-methylpiperidine was advocated, as it gave least wrong-way opening in dichloromethane and also gave rise to a low level of racemization. It was emphasized that anhydrous solvents are not required and that DMF–water (4:1) frequently gives the same yield as when anhydrous DMF is used as solvent. Also, it was found that water content did not influence the degree of urethane formation.

The use of carbonyl di-imidazole in the presence of HOBt as a coupling reagent has been recommended.[63] As carbonyl di-imidazole and HOBt were used in a 1:2 ratio, it would seem likely that carbonyldioxydibenzotriazole (22) was the actual coupling reagent. Indeed, this very compound (22) has been prepared by a reaction of trichloroacetyl chloride with two equivalents of HOBt.[64] In this case the reagent and an *N*-protected amino acid were stirred together for one hour at 0 °C in pyridine–dichloromethane to form the active ester, which was then reacted with the amino component. The substituted hydroxybenzotriazole

[62] F. M. F. Chen, R. Steinauer, and N. L. Benoiton, *J. Org. Chem.*, 1983, **48**, 2939.
[63] R. Pipkorn, M. Schmid, K. Weigand, and C. Birr, *Int. J. Pept. Protein Res.*, 1983, **21**, 100.
[64] M. Ueda, H. Oikawa, and T. Teshirogi, *Synthesis*, 1983, 908.



(22)



(23)

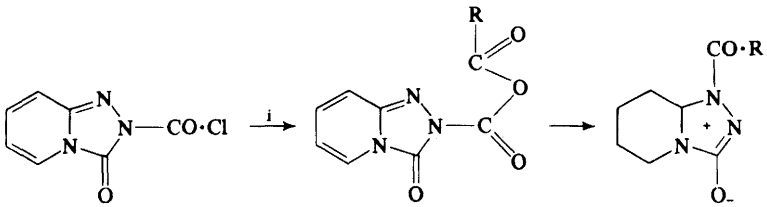
$[(Me_2N)_3\overset{+}{P}Cl]Cl^-$

(24)

reagent (23)[65] and the compound (24) have been used in the presence of HOBt;[66] in both cases no major advantage was obvious.

Mesoionic triazolopyridine derivatives (27)[67] (see Scheme 8) have been used to form peptide bonds. The compound (25) was converted to a mixed anhydride by reaction with an *N*-protected amino acid (RCO_2H), and the intermediate mixed anhydride (26) then lost carbon dioxide to give the mesoionic compound (27). This compound (27) was then reacted with an amino component to give a dipeptide. High yields were achieved, and the Izumiya test showed 6.7% racemization. The same general approach could be used to introduce urethane-protecting groups, as indicated in Scheme 9. Here the compound (28) was reacted with phosgene to give an acid chloride, which was then reacted with the corresponding alcohol to give the *N*-acyl mesoionic triazolopyridine derivative (29).

The related compound *N,N'*-carbonyldi-1,2-benzisoxazol-3-(2H)-one (30) has been prepared by reaction of 1,2-benzisoxazol-3-ol with trichloromethyl

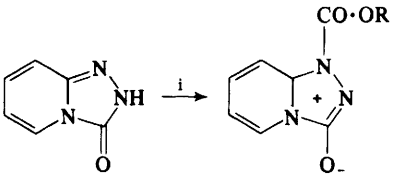


(25) (26) (27)

Reagent: i, RCO_2H/NEt_3

Scheme 8

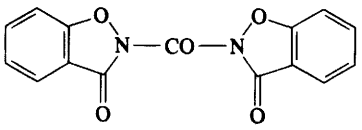
[65] M. Furukawa, N. Hohama, and T. Okawara, *Synthesis*, 1983, 42.
[66] R. Appel and E. Hiester, *Chem. Ber.*, 1983, **116**, 2037.
[67] A. Saito and B. Shimizu, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2974.
[68] M. Ueda, H. Oikawa, N. Kawaharasaki, and Y. Imai, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2485.



(28) (29)

Reagents: i, (*a*) $COCl_2$, (*b*) Py/ROH (R = Bu^t, Bzl, CCl_3, or MeO — C$_6$H$_4$ — CH_2^-)

Scheme 9



(30)

chloroformate in toluene,[68] and it was used as a coupling reagent in pyridine in a two-stage reaction, giving a good yield with virtually no racemization.

Using the Ugi four-component compensation method, peptides containing dipropyl glycine have been prepared.[69,70] Tripeptides and higher oligomers were prepared, with 4-heptanone as one of the components.

Cyclic peptides have been prepared by many workers, and it now appears that cylization using DPPA in DMF is a particularly simple method of cyclization.[71] In this example the cyclization was confirmed by analysis using fast-atom-bombardment mass spectrometry.

In cyclization studies with a model pentapeptide[72] it was found that only traces of cyclic product could be obtained from DCCI, DCCI–HOBt, IIDQ, or *O*-phenylenephosphochloridite, whereas the azide method gave a fair yield with relatively low racemization (40%) and the nitrophenyl active-ester method gave a better yield but more racemization (57%). The paper concludes that there are no general rules for choosing a method of cyclization but that separate steps of activation, followed by cyclization, are to be preferred.

Racemization. – Very little work has been carried out in the review period on racemization during peptide synthesis. Possibly this is as a result of the many careful studies over recent years that have lead to the optimization of conditions leading to minimization of racemization. In a study of the effects of additives and bases on racemization[73] it was confirmed that HOBt depressed racemization

[69] P. M. Hardy and I. N. Lingham, *Int. J. Pept. Protein Res.*, 1983, **21**, 392.
[70] P. M. Hardy and I. N. Lingham, *Int. J. Pept. Protein Res.*, 1983, **21**, 406.
[71] J. D. Young, C. E. Costello, A. Van Langenhove, E. Haber, and G. R. Matsueda, *Int. J. Pept. Protein Res.*, 1983, **22**, 374.
[72] A.-X. Ji and M. Bodanszky, *Int. J. Pept. Protein Res.*, 1983, **22**, 590.
[73] S. Isokawa, H. Arai, and M. Narita, *Nippon Kagaku Kaishi*, 1983, 124.

considerably but that HONSu only caused slight depression of racemization. When triethylamine was used in six-fold excess, racemization increased, whereas with *N*-methylmorpholine it was not observed, even at a six-fold excess of base.

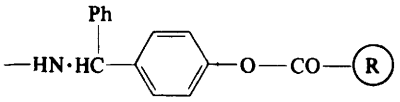
Although on many occasions h.p.l.c. is now able to resolve diastereoisomeric peptides, g.l.c. methods using a chiral support continue to provide the best means of determining the extent of racemization. An example of this is provided in a synthesis of the 46–70 fragment of human pro-insulin.[74] In this synthesis the g.l.c. racemization tests on a chiral phase showed that 4.7% racemization occurred on coupling at valine; slight racemization was also observed at lysine and glutamic acid.

Repetitive Methods of Peptide Synthesis. – *Solid-phase Synthesis.* Polyamide- and polystyrene-based resins have been used extensively in peptide synthesis. Both methods have been used in highly satisfactory syntheses, and it appears that both techniques can give very satisfactory results, provided that careful monitoring is employed during the synthesis. An example of such monitoring is provided in a synthesis of an analogue of pepsinogen 1–12.[75] In this work solid-phase sequencing and an h.p.l.c. method were used to check the homogeneity of the peptide as it was assembled on the resin.

The formation of failure sequences during solid-phase synthesis has been investigated in detail.[76] Frequently it has been assumed that failure sequences result from a lack of accessibility to the resin by the acylating species. However, it has been shown that such failure sequences are more likely to result from impurities in reagents than from the nature of the support. Failure sequences were ascribed to the presence of terminal s-butyloxycarbonyl groups on the extending *N*-terminal amino acid residue. Either these could have arisen from a pre-existing contaminant in the commercial t-butyloxycarbonylamino acid, or the s-butyloxycarbonyl group could have been obtained by trapping of the rearranged carbonium ion intermediate that is produced on acidolysis of the t-butyloxycarbonyl group. In practice, it was found that the most likely source was impurity in purchased reagents and that ultimately the t-butanol used in the preparation of the symmetrical carbonate was the source of the s-butyl contamination. Careful quality control of Boc-amino acids was therefore suggested.

The traditional chloromethylpolystyrene resin was used on many occasions. Two unusual examples are provided in the use of this solid-phase support for the synthesis of dehydro-leucine and -proline analogues of locust adipokinetic hormone[77] and in the synthesis of human β-lipotropin.[78] In the fragment assembly used in the latter synthesis Boc-glycine was attached through a thioester linkage to the resin. Following the fragment assembly, the 1–60 β-LPH was coupled with the 61–89 fragment by treatment with silver nitrate in the presence of HONSu.

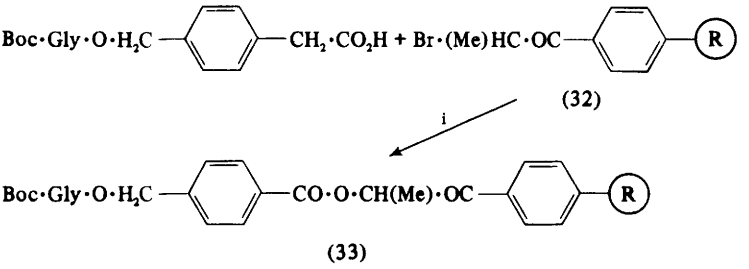
[74] V. K. Naithani and E. Schwertner, *Hoppe-Seyler's Z. Physiol. Chem.*, 1983, **364**, 1603.
[75] B. M. Dunn, M. Lewitt, and C. Pham, *Biochem. J.*, 1983, **209**, 355.
[76] S. B. H. Kent and R. B. Merrifield, *Int. J. Pept. Protein Res.*, 1983, **22**, 57.
[77] P. M. Hardy and P. W. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1983, 723.
[78] J. Blake and C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 1556.



(31)

Multi-detachable resins have been explored on several occasions, and a multi-detachable benzhydrylamine resin of type (31)[79] was used in a synthesis of (Phe^2)-Cecropin A. This synthesis, which used cyclohexyl esters of glutamic and aspartic acid for side-chain protection, gave some problems, as the solid support was found to be slightly unstable to HOBt. This instability arose from cleavage of the phenyl ester linkage by HOBt, and thus the HOBt concentration should be kept to a minimum in syntheses using this type of resin. Final deprotection at the end of the synthesis employed the 'low–high' HF procedure.

The synthesis of the protected tridecapeptide (56–68) of the V_H domain of mouse myeloma immunoglobulin M603 using two multi-detachable resins has been explored.[80] In the first synthesis 4-bromomethyl-3-nitrobenzamidomethyl resin was used, thus allowing photolytic cleavage of the protected fragment. The 2-(4-bromomethyl)phenylacetoxypropionyl resin may also be similarly used. Peptide assembly can be carried out on the support (32) by reaction with Boc-glycine-oxymethylphenylacetic acid (Boc-Gly-OMPA), according to Scheme 10. The so-called Boc-Gly-O-Pop resin (33) can then be extended to form fragments. Peptide fragments that have been cleaved from the two multi-detachable resins mentioned above may be used in a similar way as they are liberated as their OMPA acids. Such a method allows purification of the protected peptide OMPA and allows purification at an intermediate stage, followed by reattachment to the resin and further fragment assembly. The reattachment principle was illustrated by the 56–68 fragment of an immunoglobulin that was assembled on both the original multi-detachable resins with the tridecapeptide OMPA acid being reattached to various resins in good yield.



(33)

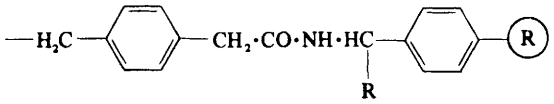
Reagent: i, KF

Scheme 10

[79] D. Andreu, R. B. Merrifield, H. Steiner, and H. G. Boman, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 6475.

[80] C. Voss, R. Dimarchi, D. B. Whitney, F. S. Tjoeng, R. B. Merrifield, and J. P. Tam, *Int. J. Pept. Protein Res.*, 1983, **22**, 204.

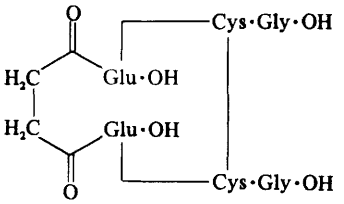
In recent years resins with increased acid stability have been developed. The PAM resin (34a) has been used on several occasions for lengthy syntheses.[81,82] A good example is provided by the synthesis of human 1–84 parathyroid hormone.[81] The C-terminal residue was attached *via* its active ester, and all other amino acids were incorporated by a double coupling cycle using DCCI. Deprotection steps were monitored qualitatively using the Kaiser test. Interesting features of the synthesis were that it utilized N^{in}-formyltryptophan and Aoc-Arg(Tos)-



(34) a; R = H (Pam resin)
b; R = Ph (Pab resin)

OH. The latter reagent was used because the Aoc derivative was found to have solubility characteristics superior to those of the corresponding Boc derivative. A final yield of 12% of 100% biologically active material was achieved after exhaustive purification. The PAM resin employed had a low substitution level, and the physical characteristics did not change during the assembly of the peptide.

A similar 1% crosslinked PAM resin was used for the synthesis of an oxidized cyclic glutathione analogue.[83] The analogue (35) was synthesized starting from Boc-Gly-OMPA. When the linear chains had been assembled on the resin they were deprotected using trifluoroacetic acid and treated with half a mole of succinic anhydride. This gave a half-amide acid, which was then reacted with DCCI–HOBt to crosslink the chains. Deprotection with HF in the presence of *p*-cresol and *p*-thiocresol gave the free peptide. During assembly the cysteine residues were protected by the 4-methylbenzyl group. After deprotection with HF, reduction followed by oxidation gave a 99% yield of the final oxidized product. The success of the synthesis depends on each resin-bound chain react-

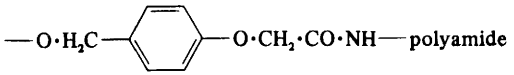


(35)

[81] T. Fairwell, A. V. Hospattankar, R. Ronan, H. B. Brewer, jun., J. K. Chang, M. Shimizu, L. Zitzner, and A. C. Arnaud, *Biochemistry*, 1983, **22**, 2691.
[82] L. Nadasdi, D. Yamashiro, C. H. Li, and J. Izdebski, *Int. J. Pept. Protein Res.*, 1983, **21**, 364.
[83] K. K. Bhargava, V. K. Sarin, N. LeTrang, A. Cerami, and R. B. Merrifield, *J. Am. Chem. Soc.*, 1983, **105**, 3247.

ing with an amino group on the same bead; thus the intra-site acylation reaction showed no reaction between beads when the low-crosslinked polymer was used. It was indicated that nearly all the functional sites have sufficient mobility to encounter a reactive site within the same bead, thus allowing a movement of approximately 200 Å. The further utility of the PAM resin is demonstrated in a synthesis of equine β-melanotropin analogues.[82]

The related PAB resin (34b) was used in the synthesis of histone fragments,[84] and once again the increased acid stability led to clean products. In part of this work the 2,6-dichlorobenzyltyrosine derivative was used, and at the end of the synthesis some 7–8% of 3-(dichlorobenzyl)tyrosine, the rearranged product, was present in the peptide as isolated by Biogel P2 chromatography. The use of cyclohexyl ether protection for tyrosine gave an improved product.



(36)

Detailed accounts of the development of the Fmoc/polydimethylacrylamide support approach have been published.[85–87] In a synthesis of human β-endorphin,[85] using a polydimethylacrylamide support and the phenoxyacetic acid linkage (36), it was found that Boc/benzyl protection gave a lower yield with aggregation during the assembly, whereas with Fmoc/butyl methodology a better yield was obtained. The use of dimethylaminopyridine as a catalyst for use during the incorporation of the first residue is cautioned, as it was found that racemization can readily occur. Synthesis using the Fmoc/polyamide approach was also demonstrated in a synthesis of enkephalin;[88] here it was found that with the linker (36) the *N*-protected amino acid caesium salt could be incorporated using DMF at room temperature in the presence of 18-crown-6, whereas with a polystyrene resin a temperature of 55 °C was required in order to achieve a good level of incorporation. In a synthesis of des(Trp^{1},Leu^{12})-minigastrin[86] the same procedure was used. However, on this occasion cleavage from the resin was brought about by aminolysis. During the build-up of the peptide chain a six-fold excess of symmetrical anhydride was used. The ninhydrin reaction was used to check the completeness of the acylation, although it was found that no repeat acylations were required. Amino acid analyses were also taken at intervals to ensure that there were no major departures from the anticipated figures. In this synthesis the acid labile protecting groups that were used for side-chain protection were removed before cleavage of the resin linkage.

[84] E. Giralt, D. Andreu, P. Miro, and E. Pedroso, *Tetrahedron*, 1983, **39**, 3185.
[85] E. Atherton, M. Caviezel, H. Fox, D. Harkiss, H. Over, and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1983, 65.
[86] E. Brown, R. C. Sheppard, and B. J. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1983, 75.
[87] E. Brown, R. C. Sheppard, and B. J. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1161.
[88] R. Colombo, E. Atherton, R. C. Sheppard, and V. Woolley, *Int. J. Pept. Protein Res.*, 1983, **21**, 118.

In the synthesis of (Leu[15])-minigastrin[86] it was emphasized that during the incorporation of the first residue (phenylalanine) using dimethylaminopyridine it was necessary to use Boc protection rather than the usual Fmoc as the Fmoc protecting group is slightly labile to DMAP. The Boc group was then removed using HCl in acetic acid, and the next fifteen residues were incorporated as Fmoc symmetrical anhydrides. Once again, the completeness of acylation was checked using ninhydrin. As the peptide terminated in N-terminal pyroglutamic acid the N-terminal residue was incorporated as Fmoc-glutamine using the nitrophenyl ester in the presence of HOBt, with a view to converting the liberated glutamine to pyroglutamic acid after deprotection. This cyclization could be achieved under carefully monitored conditions, using weak-acid catalysis.

The effect of piperidine on β-benzyl aspartyl peptides has been studied,[89] as there is a possibility that during piperidineolysis of Fmoc protection some α,β-transpeptidation may occur. In a model system it was found that during the synthesis of the hexapeptide (37) carrying a ^{14}C label in the β-benzyl aspartic acid up to 6.7% of the benzyl ester was lost during a deprotection cycle using 55% piperidine. It was also found that the reaction on a solid-phase support was slower than in solution.

Ac-Gly-Asp(O*Bzl)-Phe-Val-Gly-Ala-OH

(37)

A range of poly-*N*-acrylylpyrrolidine and poly-*N*-dimethylacrylamide polymers have been investigated as solid-phase supports.[90] It was found that these new polymers, which were crosslinked with *N,N'*-bis(acrylyl-1,2-diaminoethane), showed a better incorporation of the first residue than when the crosslinking was achieved using divinylbenzene.

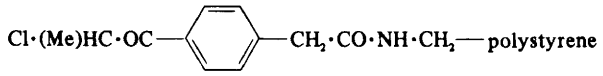
In a synthesis of a cyclic LHRH analogue[91] poly-*N*-acrylyl pyrrolidine was used as a support. In this work 4-oxymethylbenzoic acid was used as the linkage agent with an incorporated norleucine as an internal standard. Part of the synthesis was carried out with Boc for N^{α}-amino protection, but the last five residues were incorporated as their Fmoc derivatives. After removal from the resin and deprotection of side-chain benzyl-based protecting groups with trifluoromethane sulphonic acid thioanisole, the peptide was cyclized with DPPA; after the cyclization the tosyl protection was removed from arginine by treatment with HF in anisole.

A new phenolic poly-(*N*-2-methoxyethyl)acrylamide-based support has been used in the synthesis of three fragments of human β-endorphin.[92] This support is highly expanded in most common organic solvents and permits removal of the assembled protected peptide as the corresponding hydrazide by treatment of the phenyl ester linkage with hydrazine. The synthesis was carried out using

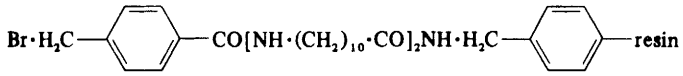
[89] I. Schon, R. Colombo, and A. Csehi, *J. Chem. Soc., Chem. Commun.*, 1983, 505.
[90] K. Varadarajan and D. Fiat, *Biopolymers*, 1983, **22**, 839.
[91] C. W. Smith, G. Skala, and J. W. Wilks, *Int. J. Pept. Protein Res.*, 1983, **21**, 127.
[92] R. Epton, G. Marr, and G. A. Willmore, *Int. J. Biol. Macromol.*, 1983, **5**, 135.

DMA as solvent, and the first residue was incorporated by treatment of the resin with a five-fold excess of the Boc-amino acid in the presence of dimethylaminopyridine and di-isopropyl carbodi-imide. Further residues were incorporated using the same di-imide in the presence of HOBt to achieve preactivation prior to acylation. Intermediate deprotection of the Boc function was achieved using BF_3 (10%) in benzyl alcohol. It was claimed that the HOBt ester generated during the preactivation procedure prevents unwanted loading of the resin, as this ester is a poor *O*-acylating reagent. After assembly a 2% solution of hydrazine hydrate in DMA cleaved the assembled peptides from the resin in two minutes. This contrasts markedly with the requirements when using a methyl or benzyl ester, where a large excess of hydrazine is required over 2–4 days.

Development of new or unusual solid-phase supports has continued. A new multi-detachable photolabile support (38) has been developed[93] and used in the synthesis of some fragments of thymopoietin(II). Protected peptides may be released by irradiation at 350 Å, and it is claimed that few side reactions occur. These phenacyl esters are very stable to anhydrous HF and to reflux in TFA. However, they are readily cleaved under nucleophilic conditions using ammonia in methanol and hydrazine, thiophenoxide, or KCN in the presence of 18-crown-6.



(38)



(39)

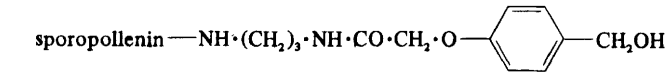
A modified Merrifield resin of structure (39) has been prepared,[94] and it is claimed that this resin, which contains a long spacer arm, has increased stability to acidolysis owing to the presence of an electron-withdrawing carbonyl group adjacent to the aromatic ring. The use of this resin support has been demonstrated in the synthesis of some fragments of apolipoprotein A2.

A macroporous Teflon-grafted polystyrene resin that is claimed to be superior to the straightforward Merrifield resin has been prepared.[95] In the synthesis of the (57–69) fragment of human haemoglobin β-chain various unusual techniques are employed, including the use of various acids in urea in organic solvents for removal of intermediate Boc protecting groups. Also, a variety of coupling methods were used including DCCI–HOBt, the mixed anhydride method, and nitrophenyl and pentafluorophenyl active esters in the presence of HOBt. During

[93] F.-S. Tjoeng and G. A. Heavner, *J. Org. Chem.*, 1983, **48**, 355.
[94] P. K. Bhatnagar, S. J. T. Mao, A. M. Gotto, jun., and J. T. Sparrow, *Peptides*, 1983, **4**, 343.
[95] M. V. Sidorova, G. A. Zheltukhina, E. I. Filippovich, M. B. Shishova, and R. P. Evstigneeva, *J. Gen. Chem. U.S.S.R.*, 1981, **51**, 2247.

synthesis any unreacted amino groups are blocked by treatment with *p*-nitrophenyl acetate. At the end of the synthesis two purifications on h.p.l.c. were required.

More details have appeared of the use of sporopollenin as a solid-phase support.[96] The chloromethyl sporopollenin was reacted with 1,3-propylenediimine to introduce the spacer unit, and then it was acylated to give the support (40).[96] This support was used in conjunction with an Fmoc-based strategy to produce a tetrapeptide that was assembled using preformed symmetrical anhydrides. Resin cleavage on this occasion was achieved using TFA.



(40)

On two occasions Sephadex LH20 has been used as a support for solid-phase synthesis.[97,98] In the first paper[97] imidazole was used as a transesterification catalyst for the attachment of Boc-amino acid nitrophenyl active esters. Interestingly the pentachlorophenyl active esters were not suitable. In the second paper[98] acylation of the Sephadex matrix was achieved by using the Boc-amino acid in the presence of carbonyl di-imidazole. Cleavage from the resin presents some problems as saponification can be used but is not an ideal method. In a test case aminolysis in methanol gave a mixture of 78% amide and 13.5% methyl ester.[97] The support does not therefore seem highly suitable for peptide synthesis.

Resin-bound *p*-nitrobenzophenone oxime has been used as a support.[99,100] N-Protected amino acids were incorporated by reaction with DCCI in the presence of HOBt; the additive 2-(hydroxyimino-2-cyanoacetate) was also used. Fragment assembly was carried out in the normal manner, and cleavage was achieved using 1-hydroxypiperidine. The resulting 1-hydroxypiperidyl esters could be converted to free acids by reduction with zinc and acetic acid. It was noted that no racemization occurred on cleavage of the oxime ester.

The soluble support polyethylene glycol (PEG) has also been used.[101–103] The formation of secondary structure during assembly on PEG was studied by synthesizing ε-benzyloxycarbonyl-lysine homoligopeptides.[101] In the solid state β-structure was observed when four residues were attached; at thirteen residues α-helical structure was observed. However, in the structure-supporting solvent trifluoroethanol α-helical structure was apparent at the hexamer level.

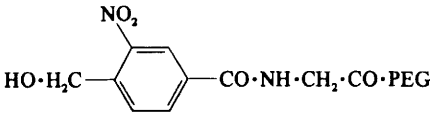
It is well known that solubility problems are frequently encountered in the synthesis of peptides of between 5 and 15 residues, and it is thought that this is

[96] R. Adamson, S. Gregson, and G. Shaw, *Int. J. Pept. Protein Res.*, 1983, **22**, 560.
[97] A. Orlowska, E. Holodowicz, and S. Drabarek, *Pol. J. Chem.*, 1981, **55**, 2349.
[98] U. O. Kalei, N. N. Podgornova, N. K. Zentsova, N. Y. Kozhevnikova, and G. P. Vlasov, *J. Gen. Chem. U.S.S.R.*, 1981, **51**, 2414.
[99] K.-S. Cheung, S. A. Wasserman, E. Dudek, S. A. Lerner, and M. Johnston, *J. Med. Chem.*, 1983, **26**, 1733.
[100] S. Hase Nakagawa and E. T. Kaiser, *J. Org. Chem.*, 1983, **48**, 678.
[101] C. Toniolo, G. M. Bonora, H. Anzinger, and M. Mutter, *Macromolecules*, 1983, **16**, 147.

due to intermolecular hydrogen bonding, giving rise to β-structure. The liquid-phase method reduces this by the PEG acting as a solubilizing macromolecular protecting group,[102] the high solubility preventing precipitation. In the present study the peptides indicated (41) were synthesized, and it was found that both had a very low solubility in chloroform. Solid-state infrared spectroscopy showed almost fully developed β-structure, which could be destroyed by addition of DMF, DMSO, or dichloromethane. It was found that DMSO was more effective than DMF at destroying the β-structure; thus in this work infrared was used as a method for quantitative estimation of the extent of β-structure development.

Boc-(Ala)$_n$-Gly-Ile-Ala$_2$-NH-PEG

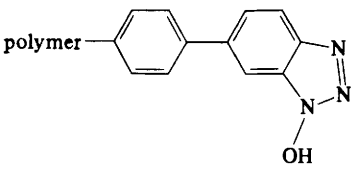
(41) n = 1 or 2



(42)

The modified PEG indicated in (42) has been used in peptide synthesis.[103] Using this linkage it was possible to assemble the bovine insulin B-chain (22–30), and the assembled fragment could be cleaved by photolysis. The photolytic cleavage yielded a material that was purified by gel filtration on Sephadex LH20.

The new polymeric hydroxybenzotriazole (43)[104] has been synthesized. This polymeric hydroxybenzotriazole has been used to form N-protected amino acid esters, which can then be used for peptide-fragment assembly. The method was illustrated by a synthesis of thymosin α1 (15–28). In this synthesis two fragments were assembled by the polymeric hydroxybenzotriazole method and then these were coupled in solution by the use of DCCI in the presence of HOBt.



(43)

[102] C. Toniolo, G. M. Bonora, M. Mutter, and F. Maser, *J. Chem. Soc., Chem. Commun.*, 1983, 1298.
[103] W. Stueber, B. Hemmasi, and E. Bayer, *Int. J. Pept. Protein Res.*, 1983, **22**, 277.
[104] M. Mokotoff and A. Patchornik, *Int. J. Pept. Protein Res.*, 1983, **21**, 145.
[105] R. Muneyuki, T. Oka, and K. Morihara, *Nippon Kagaku Kaishi (Jpn.)*, 1983, 1336.
[106] S. Fukui and A. Tanaka, *Yuki Gosei Kagaku Kyokaishi*, 1983, **41**, 384.
[107] K. Morihara, *Tanpakushitsu Kakusan Koso*, 1984, **29**, 49.
[108] P. Kuhl, J. Walpuski, and H.-D. Jakubke, *Pharmazie*, 1982, **37**, 766.
[109] M. S. Silver and S. L. T. James, *Biochim. Biophys. Acta*, 1983, **743**, 13.
[110] P. Kuhl, A. Wilsdorf, and H.-D. Jakubke, *Monatsh. Chem.*, 1983, **114**, 571.

Enzyme-mediated Synthesis and Semisynthesis. – The use of specific enzymes for the formation of amide bonds and for transpeptidation continues to be an important research area, and several reviews have appeared.[105–108]

The range of enzymes used in this work has been fairly limited, and chymotrypsin, thermolysin, pepsin, and trypsin appear to be the enzymes used predominantly. Pepsin and chymotrypsin have been used in the assembly of peptides containing phenylalanine, and much work has been done in model systems with pepsin.[109–111] In biphasic aqueous organic systems[110] with carbon tetrachloride, ethyl acetate, or n-hexane as the organic component the coupling of peptides of type (44) with phenylalanine methyl ester has been studied, and the effects of varying nucleophile and concentration have been evaluated. Under optimal conditions a reaction time of 2–6 hours could be used.

The products obtained when Z-Phe-OH was coupled to phenylalanine benzyl ester were investigated by h.p.l.c.,[111] and it was found that when the two components were coupled both Z-Phe_2-OBzl and Z-Phe_3-OBzl were produced. If the amino component was in excess then the ratio of tripeptide increased, and it was found that the total yield was less than that found when equimolar amounts of carboxyl and amino components were employed. High carboxyl component on the other hand increased the yield of dipeptide, and under these conditions a total yield of about 100% may be achieved. Similar problems were encountered when the dipeptide (45) was coupled with phenylalanine benzyl ester. Both protected Phe-Leu-Phe and Phe-Leu-Phe_2 were produced.



Z-X-Phe-OMe (44) X = Ala, Gln, or Leu



Z-Phe-Leu-OMe (45)

Chymotrypsin has also been frequently used.[112–114] The kinetics of the enzyme-catalysed synthesis of model di- and tri-peptides were examined in biphasic aqueous organic solutions,[113] and immobilized chymotrypsin was applied to the synthesis of amino acid esters.[114] A biphasic system was used with the immbolized enzyme, and in this case the yield was improved considerably by optimization of pH and by continuous extraction of the N-protected amino acid ester into the organic phase. Chloroform was found to be an ideal solvent, as the product had a high solubility, whereas pentane gave poorer yields owing to low product solubility.

Thermolysin has also been widely used,[115,116] and once again biphasic systems seem to give the best results. In a model coupling of Z-Phe-OH to leucine amide, optimal yields (91%) were obtained, using ethyl acetate/tris-malate buffer at 37 °C.[115] H.p.l.c. studies of the thermolysin-catalysed reaction between Z-X-Phe-

[111] M.-J. Tseng, S.-H. Wu, and K.-T. Wang, *Tetrahedron*, 1983, **39**, 61.
[112] E. N. Lysogorskaya, I. Y. Philippova, S. E. Boytsova, E. S. Oksenoit, L. A. Lyublinskaya, and V. M. Stepanov, *Bioorg. Khim.*, 1983, **9**, 470.
[113] P. Kuhl, J. Walpuski, and H.-D. Jakubke, *Pharmazie*, 1982, **37**, 766.
[114] J. L. Vidaluc, M. Baboulene, V. Speziale, and A. Lattes, *Tetrahedron*, 1983, **39**, 269.
[115] P. Kuhl and H.-D. Jakubke, *Z. Chem.*, 1982, **22**, 407.
[116] S. I. Wayne and J. S. Fruton, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 3241.

OH and leucine anilide indicated that a second hydrophobic residue X facilitates the reaction, and on this occasion a mixture of organic solvent and buffer was employed (20% v/v DMSO/0.5M tris-calcium acetate, pH 7.5).[116]

The use of immobilized trypsin, thermolysin, and papain was compared,[117] and in the formation of dipeptides it was found that trypsin and thermolysin were equally effective, whereas papain (a thiol proteinase) was not as effective because once the product was formed it was rapidly hydrolysed.

A further example of the use of biphasic systems is the application of carboxypeptidase-Y to the synthesis of dipeptides,[118] using a buffer/carbon tetrachloride system. In this work Z and Boc dipeptide methyl esters were coupled with valine amide and leucine amide on a preparative scale using carbon tetrachloride/carbonate as the reaction medium. The substrate-to-nucleophile ratio ranged from 1:2 to 1:3, and the yield of product was between 56 and 97%.

Insulin continues to be an active area of research, and three reviews dealing with the synthesis and semi-synthesis of insulin have appeared.[119–121]

Many semi-synthetic routes to insulin analogues involve the des-B-chain octapeptide,[119,122–124] the desoctapeptide insulin generally being prepared by treatment of porcine insulin with trypsin. The bis-Boc-desoctapeptide insulin is then prepared by treatment with di-t-butyldicarbonate, and in a study of a synthesis of analogues varying in the aromatic region B24 to B26 the bis-Boc-desoctapeptide insulin was coupled with phenylhydrazine using trypsin. The phenylhydrazide was then oxidized with BNPS skatole for 30 minutes to form the corresponding di-imide, which was used as the activating agent for the coupling of the modified octapeptide. It was found that such a mode of coupling was superior to the direct trypsin-mediated coupling of the octapeptide itself.[122] However, in work aimed at synthesizing serine B24 and serine B25 human insulin[124] it was found that the bis-Boc-desoctapeptide insulin could satisfactorily be coupled with serine B24 or serine B25 protected octapeptide, using trypsin. In order to carry out this coupling effectively the solvent mixture of DMF–butan-1,4-diol–Tris, pH 7.5, was used as the reaction medium.

Immobilized trypsin has also been used to facilitate the conversion of porcine insulin to human insulin by exchange of alanine B30 for threonine t-butyl ether, t-butyl ester.[125] A crude yield of 99% was obtained, and after removal of the t-butyl protecting groups a yield of 92% of human insulin with full potency was obtained. In this work 80–96% aqueous butandiol was used as the solvent; if

[117] A. Koennecke, M. Haensler, V. Schellenberger, and H.-D. Jakubke, *Monatsh. Chem.*, 1983, **114**, 433.
[118] P. Kuhl, N. P. Zapevalova, A. Koennecke, and H.-D. Jakubke, *Montash. Chem.*, 1983, **114**, 343.
[119] Y.-S. Zhang, *Trends Biochem. Sci.*, 1983, **8**, 16.
[120] N. Yanaihara, *Yakkyoku (Jpn.)*, 1982, **33**, 1525.
[121] D. J. Saunders, D. Brandenburg, S.-C. Chu, and C.-C. Wang, *Endeavour*, 1982, **6**, 146.
[122] M. W. Riemen, L. A. Pon, and F. H. Carpenter, *Biochemistry*, 1983, **22**, 1507.
[123] D. Cui, Q. Cao, S. Zhu, X. Zhang, and Y. Zhang, *Sci. Sin. B.*, 1983, **26**, 248.
[124] S. Shoelson, M. Fickova, M. Haneda, A. Nahum, G. Musso, E. T. Kaiser, A. H. Rubenstein, and H. Tager, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 7390.
[125] K. Rose, H. De Pury, and R. E. Offord, *Biochem. J.*, 1983, **211**, 671.

glycerol were used in place of the butandiol then the yields were halved. Replacement with one third DMSO had no effect, but the resulting solvent mixture had better solubility properties, which could well be important in the synthesis of other insulin analogues.

Recombination of the insulin A and B chains has been studied in detail.[126] It has been found that using recycling techniques the yield can be improved from 15% to around 34%. However, the yield drops after recycling five or more times, as side products accumulate owing to the alkaline destruction of cysteine and cystine during the repeated combination experiments. Such a recombination approach was used to prepare 3-iodotyrosine A19 and 3,5-di-iodotyrosine A19 porcine insulin.[127] The 3-iodotyrosine A19 and 3,5-di-iodotyrosine A19 analogues were prepared by fragment condensation following standard solution synthesis for the assembly of the fragments. During the synthesis it was found that the hydroxyl function of iodotyrosine and di-iodotyrosine did not require protection as it is believed that the bulk of the iodine atoms provide steric protection of the hydroxyl group. The synthetic modified A chain was then combined with native B chain using the intermediate tetra-*S*-sulphonate of the A chain.

In a study of the intramolecular crosslinking of insulin[128] oxalyl and malonyl bis-methionyl insulins were prepared by a reaction with the corresponding bis-nitrophenyl ester. In both cases the crosslink was formed between the A1 and B29 positions, as the amino group of phenylalanine B1 was found to be free. Treatment with cyanogen bromide in 70% formic acid in the second example removed the intramolecular crosslink to liberate the native insulin.

Ribonuclease has also been a target of semisynthesis.[129,130] Semisynthetic ribonucleases were prepared by treating *S*-protein with synthetic 1–20 *S*-peptides in which the Phe^{8}, His^{12}, and Met^{13} were retained and all other residues were replaced by alanine.[129] The syntheses of these 1–20 analogues were carried out on a PAM resin by solid-phase synthesis, and it was assumed that the alanine would induce helix formation. After combination with the *S*-protein the resulting non-covalent complexes had activity ranging from 5 to 33%. Glu^{2}, Lys^{7}, and Arg^{10} were considered in other experiments, and these were found to be responsible for the 'fine tuning' of the enzymic activity.[129] Octa-Acim-*S*-protein was also used in the preparation of alanyl-N^{ϵ}-octa-acetimidyl-*S*-protein and N^{ϵ}-octa-acetimidylribonuclease (14–124).[130]

Semisynthetic analogues of phospholipase A2 with modification at positions 6 and 7 have been prepared.[131] The method involved the preparation of des-(1–6)- and des(1–7)-acetimidyl phospholipase A2 followed by replacement with

[126] B. Schartmann, H.-G. Gattner, W. Danho, and H. Zahn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1983, **364**, 179.

[127] H.-J. Wieneke, W. Danho, E. E. Buellesbach, H.-G. Gattner, and H. Zahn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1983, **364**, 537.

[128] B. R. Srinivasa and F. H. Carpenter, *Int. J. Pept. Protein Res.*, 1983, **22**, 214.

[129] T. Kanemera, G. A. Homandberg, A. Komoriya, and I. M. Chaiken, *Int. J. Pept. Protein Res.*, 1983, **21**, 74.

[130] P. Hoogerhout and K. E. T. Kerling, *Recl. Trav. Chim. Pays-Bas*, 1983, **102**, 78.

[131] G. J. M. Van Scharrenburg, W. C. Puijk, G. H. De Haas, and A. J. Slotboom, *Eur. J. Biochem.*, 1983, **133**, 83.

synthetic 1-6 and 1-7 fragments. The resulting modified enzyme had 75% activity.

The formation of covalent hybrids from amino-terminal and carboxy-terminal fragments of ovomucoid third-domain proteins from two species has been investigated.[132] The turkey ovomucoid third domain and the Gambels-quail ovomucoid third domain were cleaved by a specific hydrolysis of the reactive-site peptide bond between the leucine[18] and glutamic acid.[19] The modified proteins were reduced with dithiothreitol, giving 1-18 and 19-56 fragments, which were separated by gel-exclusion chromatography under reducing conditions. The mixed disulphide was then formed with glutathione. The glutathione disulphide of the amino fragment from the turkey protein and the glutathione-protected carboxyl fragment of the quail protein were mixed to form the intrachain disulphides of a 'turbel' hybrid in 20% yield. The active-site leucine[18]–glutamic acid[19] peptide bond was then reformed using proteinase K to give the 'turbel'-hybrid third domain. The sequence was confirmed, and the method is claimed to be a useful procedure for preparing avian third-domain variants.

Purification Methods. – Many papers concerned with the purification of protected and deprotected peptides have appeared. There have been many developments in h.p.l.c., and it is particularly interesting to note that α- and β-isomers of aspartyl peptides in the δ-sleep-inducing peptide series could be separated by this technique using aqueous methanol as the mobile phase.[133] Also of interest is the fact that TFA has a major influence on the retention times of histidine-containing peptides on reversed-phase h.p.l.c.[134] This is due to ion pairing between TFA and the basic imidazole function of histidine. H.p.l.c. has been routinely applied to the separation of β-endorphins, but chromatography using a new crosslinked agarose of 20–40 micron in diameter[135] enables the separation of peptides of moderate size differing only in one residue. In this case partition in a two-phase system (high-performance partition chromatography) allows separation of very similar peptides, and the separation has been found to be dependent on the magnitude of the free energy of transfer from one of the phases to the other. Large free-energy differences were encountered when the residues Trp, Phe, Tyr, Leu, Ile, Met, Val, Gln, and Asn were under consideration, and with these residues separations could frequently be achieved on Sephadex G50 on a column of approximately 500 plates. However, small ΔG values are encountered with the amino acids Ala, Pro, Gly, Ser, and Thr, and in these cases the separation is more difficult, requiring the new high-performance partition chromatography on columns of several thousand plates.

Many other chromatographic techniques have been employed for the separation of peptides and diastereomers, and owing to the large number of papers in this category a summary is provided in Appendix III (Section 6).

[132] M. Wieczorek and M. Laskowski, jun., *Biochemistry*, 1983, **22**, 2630.
[133] H.-L. Jin, Y. Jin, C.-X. Li, Y.-H. Yieh, A.-H. Chi, Y.-J. Lu, C. Zhao, A.-Z. Jin, and C.-Y. Hsing, *Chromatographia*, 1983, **17**, 205.
[134] A. Seetharama Acharya, A. Di Donato, B. N. Manjula, V. A. Fischetti, and J. M. Manning, *Int. J. Pept. Protein Res.*, 1983, **22**, 78.
[135] D. Yamashiro, *Int. J. Pept. Protein Res.*, 1983, **22**, 381.

3 Syntheses

The number of synthetic peptides prepared this year has again increased, and solution synthesis is still frequently used to prepare large amounts of peptides in spite of major advances in solid-phase methodology. Peptides containing modified amide bonds are assuming increased importance, as is the synthesis of glycopeptides. Details from many of the syntheses have been discussed in the preceeding sections, and in this section only a small number of syntheses will be mentioned. The remainder are included in Appendix I (Section 4).

Some regular polymeric peptides have been prepared, and the majority of these are included in Appendix I. An interesting crosslinked block copolymer was, however, prepared using the sequences (46) and (47).[136] These fragments were assembled by the nitrophenyl active-ester method and were finally cross-linked through the side chain of lysine and glutamic acid by treatment with a water-soluble carbondi-imide. The product crosslinked polypeptide was a model of elastin, and ^{13}C n.m.r. and scanning-electron micrographs of the polymer were studied.

H-(A-Pro-Gly$_2$)$_n$-Val-OMe
(46) A = Val or Lys

H-(B-Pro-Gly$_2$)$_n$-Val-OMe
(47) B = Val or Glu

cyclo(X-Pro-D-Gln)$_2$
(48) X = Gly, Ala, Leu, Phe, or Val

cyclo(Pro-Phe-D-Trp-X-Thr-Phe)
(49) X = Lys, thiaLys, or *N*-F-Lys

Cyclic peptides also provide an interesting area, and increasingly diphenylphosphoryl azide has been used in cyclization.[71,137] In the case of peptides of type (48) cyclization of the hexamer using DPPA proved to be by far the most successful method, the peptide being activated in a concentrated solution then cyclized in dilute solution by the addition of base. The usual azide method starting from the corresponding hydrazide was also examined.

The azide method of cyclization was also used in syntheses of somatostatin analogues (49), which were prepared with a view to defining the role of the lysine at position 9.[138] The linear sequences were prepared by solid-phase synthesis and cleaved from the resin with hydrazine; cyclization was then achieved by the routine azide method using isoamyl nitrite.

An interesting application of the straightforward Merrifield solid-phase approach using chloromethyl resin was the synthesis of the 45-residue rag-weed allergen Ra-5.[139] The linear sequence containing four disulphides was prepared using the 4-methylbenzyl group for cysteine protection. DCCl couplings were used throughout with a five-fold excess of the Boc-amino acid being used.

[136] R. S. Rapaka, K. Okamoto, M. M. Long, and D. W. Urry, *Int. J. Pept. Protein Res.*, 1983, **21**, 352.
[137] K. D. Kopple and K. N. Parameswaran, *Int. J. Pept. Protein Res.*, 1983, **21**, 269.
[138] R. F. Nutt, D. F. Veber, P. E. Curley, R. Saperstein, and R. Hirschmann, *Int. J. Pept. Protein Res.*, 1983, **21**, 66.
[139] A. M. Choudhury and L. Goodfriend, *J. Protein Chem.*, 1983, **2**, 321.

N-Acetylation was used at every step with acetic anhydride and *N*-methylmorpholine to block any unreacted amino groups. This technique was still used when a negative Kaiser test was found. Removal of benzyl-based side-chain protection with HF was followed by purification on Sephadex G50, followed by disulphide formation in the presence of oxidized dithiothreitol. The synthetic rag-weed allergen was shown to be identical to the native allergen by a variety of physical and biological techniques.

Another interesting piece of work involved the synthesis of parallel and antiparallel disulphide dimers of human fibrinogen 6–14.[140] In this work Acm and *p*-methoxybenzyl were used for cysteine protection, allowing the preparation of both parallel and antiparallel disulphides. HF was initially used to remove the methoxybenzyl group, and the liberated thiol was oxidized with $K_3Fe(CN)_6$. Formation of the second disulphide bridge was then achieved by oxidation of the bis-Acm peptide with iodine.

The porcine intestinal peptide PHI and its Gln^{24} analogue have been prepared by conventional solution peptide synthesis.[141] The work employed butyl-based side-chain protection including the use of adamantyloxycarbonyl, benzyloxycarbonyl, and Nps for N^{α}-amino-group protection. Fragment condensations were carried out using DCCI in the presence of HOSu or HOBt. Problems arose in the synthesis of the C-terminal pentapeptide (50); at the tetrapeptide stage, as pyroglutamyl formation was observed, this was solved by a change in tactics whereby a 2 + 3 condensation was used. The Nps group was removed by a hydrogen halide in the presence of 2-methylindole. This lowers the acidity of the system and prevents partial acidolysis of acid labile groups. The Nps protection was also removed by tributyl phosphine. It is interesting to note that trifluoroethanol was used as a solvent for hydrogenolysis, with palladium charcoal on several occasions. In the fragment condensations HOBt gave slightly increased racemization at Phe^{6}, when compared to HONSu, this finding being similar to that observed in work on secretin.

Z-Leu-Gln-Ser(Bu^t)-Ile-Leu-NH_2

(50)

The synthesis of immunologically active peptides is an active research area, and syntheses of IgG (335–344),[142] thymopoietin (32–36),[143] and calf β-thymosin[144] have been reported using solution methods. In the synthesis of thymopoietin (32–36) benzyloxycarbonyl and adamantylmethylethoxycarbonyl (Adpoc) were both used for α-amino-group protection. The Adpoc group could be cleaved with 3% TFA in dichloromethane, leaving side-chain t-butyl protection intact.[143] The total synthesis of the 39-residue peptide calf β_8-thymosin[144]

[140] P. D. Hoeprich, jun. and R. F. Doolittle, *Biochemistry*, 1983, **22**, 2049.
[141] L. Moroder, W. Goehring, P. Lucietto, J. Musiol, R. Scharf, P. Thamm, G. Bovermann, E. Wuensch, J. Lundberg, K. Tatemoto, and V. Mutt, *Hoppe-Seyler's Z. Physiol. Chem.*, 1983, **364**, 1563.
[142] J. Martinez, J. Laur, and F. Winternitz, *Int. J. Pept. Protein Res.*, 1983, **22**, 119.
[143] W. Heinzel, T. Kronbach, and W. Voelter, *Z. Naturforsch., Teil B*, 1982, **37**, 1652.

involves fragment condensation of four fragments by the azide method, the final deprotection being by the HF procedure.

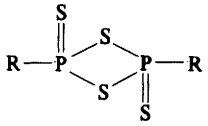
There has been an upsurge in work on glycopeptides, and several compounds related to glycophorin have been synthesized,[145,146] as have galactosyl *O*-glycopeptides.[147] Clearly, when both serine and *O*-glycosyl serine appear in the same peptide, use of *O*-glycosylated amino acid is the only means of synthesis. Base labile protecting groups, particularly Fmoc, have been used,[145,147] and the triphenylphosphine ethoxycarbonyl protecting group (Peoc) has also been employed for amino-group protection.[148,149] In both cases removal by diethylamine in t-butanol or morpholine in dichloromethane leaves the glycosidic linkage unaffected.

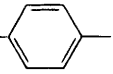
Protection of the carboxyl function of amino acids in glycopeptide synthesis has also been considered, and the 2-bromoethyl esters of serine have been used.[150] Thus, Z-Ser-bromoethyl ester can be converted to the glycoserine derivative by treatment with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide. The carboxyl protecting group can then be removed by initial treatment with sodium iodide in acetone to convert the bromoethyl ester to the iodoethyl ester, which is then treated with zinc to effect deprotection.[150] These approaches have been used previously in more straightforward peptide chemistry.[151] Allyl esters have also been used in glycopeptide work;[152] in this case the protecting group is introduced by reaction of the free amino acid with allyl alcohol in the presence of tosic acid. These allyl esters can be used in glycopeptide synthesis and are stable to acid, but they are readily cleaved by treatment with Tris-triphenylphosphine rhodium(I) chloride in ethanol–water (9:1), leaving amino protecting groups and the *N*-glycosidic bond unaffected.

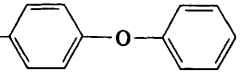
Use of Lawesson's reagent (LR) (51a) has prompted an increase in work on endothiopeptides. Simple endothiopeptides[153] thioaspartam[154] and endothionated melanostatin[155] have been prepared. The replacement of an amide bond by a thioamide bond has been shown to cause little difference in geometry, except for the increase in bond length on moving from a carbon–oxygen to a carbon–sulphur bond length.[156]

Standard coupling and deprotection sequences allowed tripeptides with specific thioamide-bond replacement to be prepared.[153] Benzyloxycarbonyl could

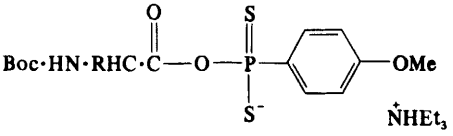
[144] T. Abiko and H. Sekino, *Chem. Pharm. Bull.*, 1983, **31**, 1320.
[145] B. Ferrari and A. A. Pavia, *Int. J. Pept. Protein Res.*, 1983, **22**, 549.
[146] B. Ferrari and A. A. Pavia, *Int. J. Pept. Protein Res.*, 1983, **22**, 539.
[147] J. M. Lacombe and A. A. Favia, *J. Org. Chem.*, 1983, **48**, 2557.
[148] H. Kunz and H. Kauth, *Liebigs Ann. Chem.*, 1983, 337.
[149] H. Kauth and H. Kunz, *Liebigs/Ann. Chem.*, 1983, 360.
[150] M. Buchholz and H. Kunz, *Liebigs Ann. Chem.*, 1983, 1859.
[151] M. Brenner and W. Huber, *Helv. Chim. Acta*, 1953, **36**, 1109.
[152] H. Waldmann and H. Kunz, *Liebigs Ann. Chem.*, 1983, 1712.
[153] D. W. Brown, M. M. Campbell, and C. V. Walker, *Tetrahedron*, 1983, **39**, 1075.
[154] B. Yde, I. Thomsen, M. Thorsen, K. Clausen, and S.-O. Lawesson, *Tetrahedron*, 1983, **39**, 4121.
[155] M. Thorsen, B. Yde, U. Pedersen, K. Clausen, and S.-O. Lawesson, *Tetrahedron*, 1983, **39**, 3429.
[156] T. F. M. La Cour, H. A. S. Hansen, K. Clausen, and S.-O. Lawesson, *Int. J. Pept. Protein Res.*, 1983, **22**, 509.



(51) a; R =  (LR)

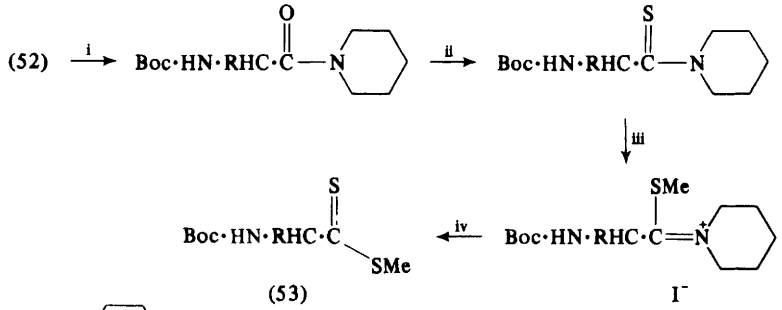
b; R = 

be removed from endothiopeptides by treatment with HBr in acetic acid, and t-butyl-based protection could be removed by TFA. Treatment with hydroxide to remove ester protection was unsatisfactory as a mixture of deep-red/orange products was obtained.[153] No racemization was encountered during the conversion of amide to thioamide bonds.[153,155] In the synthesis of endothionated melanostatin[155] Lawesson's reagent was used as both the thionation reagent and the coupling reagent, as Boc-amino acids may be activated with the formation of compounds of the type (52). Treatment of these intermediates with an



(52)

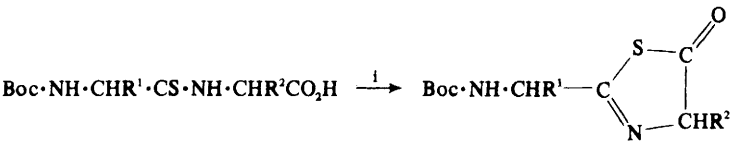
amino component gives the normal peptide bond, whereas reaction according to Scheme 11 allows the methyl thioester (53) to be prepared, which can be used directly in coupling reactions.



Reagents: i,  ; ii, (51a)/80 °C; iii, MeI/THF; iv, H_2S

Scheme 11

A major disadvantage of (51a) as a thionating reagent lies in the fact that many protecting groups are thermally unstable, and in order to use the reagent a temperature of 80 °C is employed. There is also a lack of regioselectivity in tri- and higher peptides. It is claimed that the related compound (51b)[157] is readily soluble in THF, allowing thionation between 0 and 25 °C. Also, it shows a high degree of regioselectivity being considerably dependent on the steric environment around each amide bond. It was found that an adjacent glycine residue gave a rapid and selective reaction, whereas large flanking side chains or proline encouraged a much slower reaction. Elongation of Boc monothiopeptides can be achieved by removal of the Boc protection with acid followed by coupling in the standard way with DCCI–HOBt or EEDQ. However, if an endothiodipeptide is activated by DCCI as indicated in Scheme 12, then the corresponding diazalactone (54) is formed.[157]



Reagent: i, DCCI

(54)

Scheme 12

Retro-inverso peptides have also attracted attention. These peptides involve modification of the peptide backbone by replacement of one of the residues in the chain by a 1,1-diaminoalkane derivative, and at some point further along the chain a malonic acid residue is incorporated. The effect of this is that the intervening peptide chain has the sense of the amide bond reversed. Frequently the *gem*-diaminoalkane derivative is produced by Curtius-type rearrangement of N-protected amino acid azides.[158] Alternatively, N-protected amino acid amides may be converted to these compounds by rearrangement on treatment with bis(trifluoroacetoxy)iodobenzene.[159] Use of such diaminoalkanes requires selective protection of the two amino groups, and using this approach retro-inverso analogues of somatostatin[160] and substance P[161,162] have been synthesized.

In the work on somatostatin Fmoc and Nps were used for protection of amino termini. The malonate component was incorporated as a mono ester, and ultimately a diastereoisomeric tetrapeptide was produced that was combined with the main hexapeptide to give the fully protected somatostatin.[160] Both

[157] G. Lajoie, F. Lepine, L. Maziak, and B. Belleau, *Tetrahedron Lett.*, 1983, **24**, 3815.

[158] M. Chorev and M. Goodman, *Int. J. Pept. Protein Res.*, 1983, **21**, 258.

[159] P. V. Pallai, S. Richman, R. S. Struthers, and M. Goodman, *Int. J. Pept. Protein Res.*, 1983, **21**, 84.

[160] P. Pallai, S. Struthers, M. Goodman, J. Rivier, and W. Vale, *Biopolymers*, 1983, **22**, 2523.

[161] M. Chorev, E. Rubini, C. Gilon, U. Wormser, and Z. Selinger, *J. Med. Chem.*, 1983, **26**, 129.

[162] A. Pessi, M. Pinori, A. S. Verdini, and G. C. Viscomi, *J. Chem. Soc., Chem. Commun.*, 1983, 195.

solution- and solid-phase approaches have been used for the synthesis of retro-inverso peptides, and in the synthesis of an analogue of substance P[162] a poly-dimethylacrylamide was used as the support with chain extension using Fmoc anhydrides.

In conclusion, it is worth noting that many of the peptide modifications that are being investigated at present cannot be achieved using the methods of genetic engineering, and it is therefore probable that prospects for peptide chemists are good for the foreseeable future.

4 Appendix I: A List of Syntheses Reported in 1983

The syntheses are listed under the name of the peptide to which they relate, as in previous years.

Peptide	*Ref.*
Natural Peptides, Proteins, and Partial Sequences	
α-Factor	
α-Factor analogues of *Saccharomyces cerevisiae*	163
Adrenocorticotropic hormone (ACTH)	
[Asp^{25},Ala^{26},Gly^{27},Gln^{20}] ACTH (25–39)	47
Biotinyl derivative of ACTH	164
Tritiated ACTH (4–9)	165
Tritiated ACTH (5–7)	166
Adipokinetic hormone	
[4,5-^{3}H-Leu^2]- and [3,4-^{3}H-Pro^6]-adipokinetic hormone(locust)	167
[4,5-ΔLeu^2]- and [3,4-ΔPro^6]-adipokinetic hormone(locust)	77
Agrobactin A	
Agrobactin A	168
Alamethicin	
Alamethicin fragments	169
Amanita toxin	
Amanita toxic peptides	170

[163] P. Shenbagamurthi, R. Baffi, S. A. Khan, P. Lipke, C. Pousman, J. M. Becker, and F. Naider, *Biochemistry*, 1983, **22**, 1298.
[164] H. Romovacek, F. M. Finn, and K. Hofmann, *Biochemistry*, 1983, **22**, 904.
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Lipoprotein, N-terminal pentapeptide	282
Luteinizing-hormone-releasing hormone (LHRH)	
N-Acetyl-LHRH analogues	283
Chicken LHRH	284
LHRH antagonist	285
Tritiated LHRH	286
1,6-Cyclo[Ac-Glu^1, D-Phe^2, D-Trp^3, D-Lys^6]LHRH	91
[Glu^8]LHRH	287
[X^3]- and [X^6]-LHRH [X = β-(3-quinolyl)-D-α-alanine]	288
Lysozyme	
Lysozyme containing intramolecular crosslinkage Lys^{13}-Leu^{129}	289
Melanostatin	
Endothionated melanostatin	155
Analogues of [D-Phe^7]α-MSH substituted at residues 4 and 11	290
[Cys^4,Cys^{10}]MSH analogues	291
Position-1 analogues of β-MSH	82
Metallothionein	
Human hepatic metallothionein II fragments	292
Human metallothionein, hMT II (36–61)	39
Molluscan neuropeptide	
Molluscan neuropeptide, H-Phe-D-Leu-Arg-Phe-NH_2	16
Morphiceptin	
Morphiceptin analogues	293
Mucronin	
Mucronin B	294

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Peptide	*Ref.*
Mycobactin	
Mycobactin S2	295
Myelin basic protein	
Myelin basic protein (115–118)	296
Neokyotorphin	
Neokyotorphin	297
Neokyotorphin	40
Nisin ring	
Nisin ring A	298
Nocardicin	
Nocardicin	299
Opioid peptide E	
Opioid peptide E	32
Ovomucoid third domain	
Ovomucoid third domain analogues	132
Oxytocin	
Oxytocin antagonists	300
[5-β-Cyanoalanine]- and [9-α-aminoacetonitrile]-oxytocin	301
[Cyclo-Leu^2]- and [1-penicillamine^1,cyclo-Leu^2]-oxytocin	302
[1-Desaminopenicillamine,8-α-hydroxyisocaproic acid]oxytocin	303
[8-α-Hydroxyisocaproic acid,9-glycolic amide]oxytocin	304
[Sar^7]- and [(Me)Ala^7]-oxytocin analogues	305
PHI	
PHI	43
PHI and [Gln^{24}]PHI	141
Parathyroid hormone (PTH)	
Human (1–84) PTH	81
[Asn^{76}]PTH(human) (1–84)	36
Pepsinogen	
Pepsinogen (1–12) analogue	75

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Phalloidin	
Phalloidin	306
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Position-7 analogue of phalloidin	309
Phosphopeptides	
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Aminoalkylphosphonic acid derivatives	313
Aminoethylphosphonic ester derivatives	314
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Phospholipase	
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Phyllolitorin	
Phyllolitorin	319
Proalbumin	
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Prothrombin	
Prothrombin (202–215)	23
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Rag-weed allergen	
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Ribonuclease (RNase)	
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RNase analogues	130

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Peptide	*Ref.*
Rimorphin	
Rimorphin	44
Sauvagine	
Sauvagine (17–40)	321
Somatomedin C	
Somatomedin C, insulin-like growth factor I	322
Somatostatin	
Somatostatin	57
Extended retro-inverso analogues of somatostatin	160
Somatostatin analogues	138
Somatostatin analogues	323
Staphylococcal nuclease	
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Substance P	325
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Substance P (6–11), retro-inverso analogue	327
Substance P analogues	328
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Glycosylated analogues of the C-terminal hexa- and hepta-peptide of substance P	330
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[Hcy^{11}]substance P	35
Retro-inverso analogue of substance P	162
Retro-inverso analogue of substance P	161
Suzukacillin	
Suzukacillin (6–10)	332

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Peptide	*Ref.*
Tetaine	
Tetaine analogue	333
Thienamycin	
Thienamycin	334
Thienamycin analogues	335
Thienamycin analogues	336
Thioredoxin	
Thioredoxin model peptide	337
Thrombin	
Thrombin amino-terminal 11-peptide	338
Thymic peptides	
Serum thymic factor (FTS)	339
FTS analogues	340
FTS analogues	341
FTS analogues	342
FTS analogues	343
[^{14}C]Thymopoietin (32–36)	344
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Thymopoietin II fragments	93
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Thymosin α_1 (15–28)	104
Thymosin β_4	346
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Thyrotropin-releasing hormone (TRH)	
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Peptide	*Ref.*
5-Fluoroimidazole TRH	349
Tuftsin	
Tuftsin containing IgG fragment (285–296)	350
Four analogues of tuftsin	351
Position-4 analogues of tuftsin	352
Urotensin	
Urotensin (4–28)	353
Vasopressin	
Vasopressin analogues modified at positions 2, 3, and 8	354
[1-(β-Mercapto-β,β-cyclopentamethylenepropionic acid),D-Phe^2,Val^4]-arginine vasopressin analogues	355
[Asu^{1,6},Phe(N$_3$)^3]vasopressin	356
Des-(Gly.NH$_2$)^9,X^8 analogues of vasopressin (X = non-coded basic residue)	357
[Sar^7]- and [(Me)Ala^7]-vasopressin analogues	305
Zizyphine	
10,11-Dihydrozizyphines	358
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Dihydrozizyphine A/B	360

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Poly(β-Ala)	362
Boc-Ala-(Aib-Ala)$_3$-OMe	363

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Peptide	*Ref.*
Poly[Ala-Gly-Gln(C_3H_6OH)]	364
Boc-Ala_n-Gly-Ile-Ala_2-NHPEG (n = 1 or 2)	102
Boc-Ala_2-Pro-Gly-Ala_2NHPEG	365
Boc-X_n-OEt (n = 2–8, X = Ala, Val, or Leu)	366
Poly[Asp(OBzl)-Glu(OMe)]	367
Oligopeptide copolymers	368
Dehydro-oligopeptides	369
9-Carboxymethylcarbazole-substituted polydehydroalanine	370
Poly(β-diaminopropionic acid)	371
Oligomers of dipropylglycine	70
Poly(5-ethyl-Pro)	372
Crosslinked poly[Glu-Glu(OBzl)]	373
Poly[Glu(OBzl)]	374
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Dnp-Gly_n-D-Ala-Pro-Gly_n-pNA (n = 1–3)	377
Poly(His-Asp-Ser-Gly)	378
Crosslinked resin bound Leu_n-$(Leu_3)_n$-Phe-resin (n = 1–3)	379
Poly(Leu-Lys)	380
Poly(Lys-Ala)	381
Poly[Lys(carbazolylcarbonyl)]	382

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Peptide	*Ref.*
Poly(Lys-Gly) and poly(Lys_n-X_m) (X = Leu or Ser)	383
Poly[Lys-(Gly_2-His)]	384
PEG-bound [L-Lys(Z)]$_n$	101
Poly(methacryloyl-Ala)	385
Thyroxine–methacrylate co-polymers	386
Boc-Val-(Aib-Val)$_3$-OMe	363
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ACE inhibitor, BzNHCH(Bzl)CH_2-Gly-Pro-OH	397
ACE inhibitors, phosphonic amides and acids	398
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Peptide	*Ref.*
β-[^{3}H] Leucine	541
^{13}C-Labelled leucine	542
[^{14}C]Lys-Gly-NMePh	543
Fluorescent analogues of methotrexate	544
A tripeptide mustard	545
Labelled neuropeptide precursors	546
Nucleopeptides	547, 548
Nucleosidylamino acids	549
Palmitoyl derivatives of cysteine	550
Peptide anti-cancer prodrugs	551
Peptides containing β-pyrazinyl-L-alanine	552
Peptide phorbol esters	553
D,L-Phosphotryptophan derivatives	554
Photoaffinity label for *N*-formylpeptide receptor	555
Photoaffinity-labelled peptides	556
Retro-inverso peptides	158, 159
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Deuteriated serine derivatives	558
Oligonucleotidyl serine derivatives	559
Spin-labelled peptides	560
Thioacylamino acid esters	561

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Peptide	*Ref.*
3,5-Substituted 2-thiohydantoins	562
O-Alkyltyrosine derivatives	563
Tyrosine and glutamine peptides	564
Uridylyl (P—O) amino acids and peptides	565
Analogues of virginiamycin, cyclo[Boc-Ser-D-Abu-Pro-(Me)Phe-Pip-Phg-O]	566
Vitamin K-dependent proteins containing -Gla-Arg-Gla- unit	567
Z-Gly aromatic *o*-hydroxyoximes	568

5 Appendix II: Amino Acid Derivatives Useful in Synthesis

The list of derivatives is divided into two groups, the coded and the uncoded amino acids.

Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Coded Amino Acids					
Alanine					
Boc-Ala-OCAM	96–98	−27	2.1	DMF	21
Boc-Ala-OFm	77–79	−24	1.3	$CHCl_3$	20
Boc-Ala-OHAP	104–106	−67.1	1	$CHCl_3$	58
Boc-Ala-OHBA	100–102	−26.4	1	$CHCl_3$	58
Bu^{t}meoc-Ala-OH	112–114	−10.15	1.06	MeOH	16
$Cl^-H_2^+$-Ala-OEt(Br)	138	+2.1	4.0	DMF	150
Fmoc-Ala-OH	142–143	−19.7	1	DMF	12
Fmoc-Ala-OPfp	171–173	−22.7	1	$CHCl_3$	56
$TosO^-H_2^+$-Ala-OAll	84–85	−1.6	1	MeOH	152
Z-Ala-OCAM	60–64	−13	2.1	DMF	21
Z-Ala-OFm	90–91	−38.4	1	MeOH	19
Z-Ala-OHAP	99.5–100	−42.4	1	DMF	58
Z-Ala-OHBA	94–96	−19.7	1	DMF	58
Arginine					
Ddz-Arg-OH	oil	—	—	—	63
Ddz-Arg(Tos)-OH	128	−7.0	1	DMF	63
Fmoc-Arg(Mds)-OH	127–129	—	—	—	30
Fmoc-Arg(Mtr)-OH	68–72	—	—	—	30

*1 The optical rotations $[\alpha]_D$ were measured in the range 18–27 °C, the actual temperature being given in the original paper. *2 Optical rotation measured at 589 nm

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Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Fmoc-Arg(PMbs)-OH	123–125	–	–	–	30
Z-Arg(Mbs)-OH-DCHA	110	+2.5	1.01	MeOH	143
Asparagine					
Boc-Asn(Xan)-OH	183–185	+4.4	1	DMF	85
Fmoc-Asn-OPfp	164–165	−13.1	1	Dioxan	56
Aspartic acid					
Boc-Asp(OBzl)-NH_2	146–147	−2.8	1.01	EtOH	45
Boc-Asp(OBzl)-Pfp	155–156	−6.6	0.37	EtOH	45
Boc-Asp(O[$^{14}C^{\alpha}$]Bzl)-OH	102–103	−21.3	2	DMF	89
Fmoc-Asp(OBu^t)-OPfp	98–100	−2.5	1	$CHCl_3$	56
Fmoc-Asp(OBzl)-OH	113–115	−3.5	1	MeOH	56
Fmoc-Asp(OBzl)-OPfp	128–131	−14.0	1	EtOAc	56
Fmoc-Asp(OH)-OBzl	112–115	+4.0	1	MeOH	56
Fmoc-Asp(OPfp)-OBzl	124–125	−5.1	1	EtOAc	56
Peoc-Asp(OBzl)-OBu^t	amorph	−7.82	1.1	$CHCl_3$	148
Peoc-Asp(OBzl)-OH	amorph	+7.18	1	$CHCl_3$	148
Peoc-Asp(OH)-OBu^t	amorph	−2.22	1	$CHCl_3$	148
Troc-Asp(OBzl)-OH-Bu^tNH_2	148–149	+15.1	1	EtOH	27
Troc-Asp(OEt)-OH-Bu^tNH_2	158–159	+16.6	1	EtOH	27
Troc-Asp(OH)-OBzl-Bu^tNH_2	144–145	+3.3	1	EtOH	27
Troc-Asp(OH)-OEt-Bu^tNH_2	140–141	+2.6	1	EtOH	27
Z-Asp(ONSu)-OBu^t	89–92	+17.2	0.98	Dioxan	45
Cysteine					
Boc-Cys(Acm)-OPcp	111–114	−58.45	1	DMF	50
$Cl^-H_2^+$-Cys(Mpt)-OH	157–158	−18.4	1	MeOH	34
$Cl^-H_2^+$-Cys(Mpt)-OMe	oil	–	–	–	34
Fmoc-Cys(Acm)-OH	amorph	−34.4	1	MeOH	56
Fmoc-Cys(Acm)-OPfp	157–158	−32.6	1	$CHCl_3$	56
Fmoc-Cys(Bu^t)-OH	133–134	−10.8	1	MeOH	160
Fmoc-Cys(Bzl)-OH	125–126	−40.6	1	MeOH	56
Fmoc-Cys(Bzl)-OPfp	132–134	−31.0	1	$CHCl_3$	56
Mpt-Cys(Mpt)-OH-DCHA	150–151	−6.2	1	MeOH	34
Mpt-Cys(Mpt)-OMe	oil	−13	1	EtOH	34
Glutamine					
Boc-Gln(Xan)-OH	152–154	−9.85	1	DMF	85
Bu^{t}meoc-Gln-OH	97–108	−7.0	1.01	MeOH	16
Fmoc-Gln-OPfp	151–153	−19.8	1	Dioxan	56
Z-Gln-NH_2	195–198	+8.5	1	DMF	50

Compound	*M.p./°C*	$[\alpha]_D$*[1]	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Glutamic acid					
Boc-Glu(OMe)-ONSu	98–100	−43.4	1.6	EtOH	496
Bpoc-Glu(Bu^t)-OH-DCHA	134–136	+14.2	1.03	MeOH	85
Fmoc-Glu(OBu^t)-OPfp	121–123	−25.2	1	$CHCl_3$	56
Troc-Glu	124–125	−28.33	5.7	DMF	27
Troc-Glu(OBzl)-OH-Bu^{t}NH$_2$	153–154	+13.2	1	EtOH	27
Troc-Glu(OEt)-OH-Bu^{t}NH$_2$	155–156	−15.4	1	EtOH	27
Troc-Glu(OH)-OBzl	71.5–72	−21.9	3.5	EtOH	27
Troc-Glu(OH)-OBzl-Bu^{t}NH$_2$	162–164	−16.1	1	EtOH	27
Troc-Glu(OH)-OEt-Bu^{t}NH$_2$	155–156	−15.4	1	EtOH	27
Glycine					
Boc-Gly-NHNH-Troc	124–125	–	–	–	144
Boc-Gly-OCAM	64–67	–	–	–	21
Boc-Gly-OHAP	135–138	–	–	–	58
Boc-Gly-OHBA	123–126	–	–	–	58
Bu^{t}meoc-Gly-OEt	117–118	–	–	–	16
Fmoc-Gly-OH	173–174	–	–	–	10
Fmoc-Gly-OPfp	160–161	–	–	–	56
TosO$^-$H$_2^+$-Gly-OAll	57–58	–	–	–	152
Z-Gly-OCAM	105–108	–	–	–	21
Isoleucine					
Bz-Ile-OCAM	104–105	+11	2.2	DMF	21
Fmoc-Ile-Pfp	96–98	−13.4	1	$CHCl_3$	56
Peoc-Ile-OH	amorph	+5.1	1.15	$CHCl_3$	148
TosO$^-$H$_2^+$-Ile-OAll	91–92	+13.7	1	CH_2Cl_2	152
Z(OMe)-Ile-NH$_2$	224–225	+21.7	0.4	DMF	43
Leucine					
Boc-Leu-OFm	61–67	–	–	–	20
Boc-Leu-OPic	oil	−31	1	DMF	23
Bu^{t}meoc-Leu-OH-DCHA	113–118	−14.4	1	MeOH	16
Bz-Leu-OCAM	129–132	−7	2.4	DMF	21
Cl$^-$H$_2^+$-Leu-OEt(Br)	155	+3.9	1.1	DMF	150
Cl$^-$H$_2^+$-Leu-OEt(Cl)	146	+4.7	1.1	CH_2Cl_2	150
Fmoc-Leu-OPfp	114–116	−25.7	1	$CHCl_3$	56
TosO$^-$H$_2^+$-Leu-OAll	117–118	+5.3	1	MeOH	152
Z-Leu-OHAP	112–113.5	−36.5	1	DMF	58
Lysine					
Adpoc-Lys(Boc)-ONSu	170	−25.1	1.5	DMF	143
Boc-Lys(Teoc)-OH	oil	–	–	–	29

Compound	*M.p./°C*	$[\alpha]_D$*[1]	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Boc-Lys(Z)-OHAP	105–107	−32.9	1	DMF	58
Bu^{t}meoc-Lys(Boc)-OH-DCHA	71–74	+7.3	1.0	MeOH	16
$Cl^-H_2^+$-Lys(Boc)-OMe	165–167	–	–	–	551
Fmoc-Lys(Boc)-OPfp	89–93	−14.2	1	$CHCl_3$	56
Fmoc-Lys(Z)-OH	108–110	−2.0	1	MeOH	56
Fmoc-Lys(Z)-OPfp	106–108	−10.5	1	$CHCl_3$	56
H-Lys(Teoc)-OH	231–232	–	–	–	29
H-Lys(Z)-OBu^t	139–140	+12.3	2	EtOH	460
$TosO^-H_2^+$-Lys(Fmoc)-OBzl	104–105	–	–	–	522
Methionine					
Boc-Met-OCAM	78–81	−29	2.7	DMF	21
Boc-Met(O_2)-OH	104–107	−14.4	2	DMF	546
Fmoc-Met-OPfp	102–104	−12.6	1	$CHCl_3$	56
Peoc-Met-OH	amorph	+5.07	1.1	$CHCl_3$	148
Phenylalanine					
Adpoc-Phe-OH	106–108	−2.3	1	MeOH	16
Boc-Phe-OCAM	113–116	−16	2.95	EtOH	21
Boc-Phe-OFm	126–127	−32.9	1	$CHCl_3$	19
Boc-Phe-OFm	125–126	−6	0.84	$CHCl_3$	20
Boc-Phe-OHAP	114.5–116.5	−21.0	1	DMF	58
Boc-Phe-OHBA	113–115.5	−2.9	1	DMF	58
Bu^{t}meoc-Phe-OH	115–117	+16.31	1.04	MeOH	16
$Cl^-H_2^+$-Phe-OEt(Br)	167	+14.4	1.1	DMF	150
Fmoc-Phe-OH	184–185	–	–	–	10
Fmoc-Phe-OH	178–179	−41.7	1	DMF	12
Fmoc-Phe-OPfp	154–157	−20.3	1	$CHCl_3$	56
Nps-Phe-OHAP	132–134	−129.1	1	DMF	58
Nps-Phe-OHAP-($5NO_2$)	145.5–148.5	−90.7	1	DMF	58
$TosO^-H_2^+$-Phe-OAll	147	+2.6	1	MeOH	152
Troc-Phe-OH	126–127	−29.9	3	MeOH	12
Z-Phe-OCAM	105–108	−24	3.05	EtOH	21
Z-Phe-OFm	114–116	−21	1	EtOAc	20
Z-Phe-OHAP	116.5–118.5	−21.1	1	DMF	58
Z-Phe-ONSu	133–135	+12.0	1	MeOH	141
Proline					
Boc-Pro-OCAM	78	−54	2.05	EtOH	21
Boc-Pro-OFm	oil	−45.5	1	MeOH	19
Fmoc-Pro-OH	116–117	−33.2	1	DMF	12
Fmoc-Pro-OPfp	127–129	−59.2	1	$CHCl_3$	56
Nps-Pro-OHAP	152.5–154	−193.5	1	DMF	58
Peoc-Pro-OH	amorph	−0.5	1.25	MeOH	148

Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
$TosO^-H_2^+$-Pro-OAll	oil	−20.2	1	CH_2Cl_2	152
Serine					
Boc-Ser(Bu^t)-ONSu	119–121	−12.3	1	$CHCl_3$	145
Boc-Ser-OBzl	64–67	−17.26*	1	MeOH	566
Bu^{t}meoc-Ser(Bu^t)-OH-DCHA	70–85	+16.2	1.0	MeOH	16
$Cl^-H_2^+$-Ser-OEt(Br)	85–86	−6.2	4.3	DMF	150
$Cl^-H_2^+$-Ser-OEt(Cl)	90–92	−5.7	1.1	DMF	150
Fmoc-Ser(Bzl)-OH	118–119	+25.1	1	EtOAc	12
Fmoc-Ser-OMe	127–129	−9.6	1	MeOH	12
Fmoc-Ser-ONp	125–127	−31.1	1	$CHCl_3$	145
Fmoc-Ser-OPfp	125–130	−21.3	1	$CHCl_3$	56
Peoc-Ser(Bu^t)-OBu	amorph	+8.9	1	$CHCl_3$	148
Peoc-Ser-OH	147–152	−3.15	1	MeOH	148
$TosO^-H_2^+$-Ser-OAll	oil	−3.0	1	MeOH	152
Troc-Ser(Bzl)-OH	117–118	−2.46	5	DMF	12
Troc-Ser-OH	114–115	−16.7	5	DMF	12
Threonine					
Boc-Thr-NHNH-Troc	78–80	−15.1	1	DMF	144
Fmoc-Thr-OH	amorph	−4.8	1	MeOH	56
Fmoc-Thr-ONSu	foam	−26.5	1	$CHCl_3$	147
Fmoc-Thr-OPfp	126–128	−33.0	1	$CHCl_3$	56
Peoc-Thr(Bu^t)-OBu^t	amorph	+3.11	1.1	$CHCl_3$	148
PeocThrOH	158	−1.78	1.25	$CHCl_3$	148
$TosO^-H_2^+$-Thr-OAll	oil	−6.7	1	MeOH	152
Troc-Thr-OH-Bu^{t}NH$_2$	144–146	+4.82	5	MeOH	12
Boc-Trp(Troc)-OBzl	122–125	–	–	–	33
Tryptophan					
Boc-Trp-OCAM	154–158	−26	2	DMF	21
Boc-Trp(Troc)-OH	186–188	–	–	–	33
Boc-Trp(Z)-OMe	118–120	–	–	–	33
Bu^{t}meoc-Trp-OH	86	−13.5	1.0	MeOH	16
Fmoc-Trp-OPfp	185–186	−42.1	1	$CHCl_3$	56
H-Trp(Troc)-OH	222–225	–	–	–	33
Troc-Trp-OH	197–198	+6.58	5	MeOH	12
Troc-Trp-OH-Bu^{t}NH$_2$	104–106	−32.36	5	MeOH	12
Tyrosine					
Boc-Tyr(Bu^t)-OH	112–115	+15.86	1.03	MeOH	85
Boc-Tyr(cHex)-OH-DCHA	–	+29.5	1	MeOH	84
Boc-Tyr(cHex)-OMe	–	+98-2	1	$CHCl_3$	84
Boc-Tyr-NH$_2$	191–193	+20.1	0.98	MeOH	45
Fmoc-Tyr(Bu^t)-OPfp	76–78	−12.7	1	$CHCl_3$	56
Tfa-Tyr(Bzl)-OBzl	83–84	−15.0	1	MeOH	483

Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
$TosO^-H_2^+$-Tyr(Bu^t)-OPh	216–218	+27.1	1	DMF	50
Z-Tyr(Bu^t)-OPh	92–95	−13.1	1	DMF	50
Z-Tyr-NH_2	164–166	+2.0	1	EtOH	45
Valine					
Adpoc-Val-OH	150	−3,2	1	MeOH	143
Adpoc-Val-ONSu	128–129	−27.2	1.03	MeOH	143
Boc-Val-OFm	65–66	−37.2	1	MeOH	19
Bu^{t}meoc-Val-OH-DCHA	128–138	−9.8	1.03	MeOH	16
$Cl^-H_2^+$-Val-OEt(Br)	73–75	+8.4	1.4	DMF	150
Fmoc-Val-OH	142–143	−18.7	1	DMF	12
Fmoc-Val-OPfp	122–123	−21.9	1	$CHCl_3$	56
$TosO^-H_2^+$-Val-OAll	84–86	+7.0	1	MeOH	152

Uncoded Amino Acids
(All compounds are of the L-configuration unless specified otherwise.)

Alanine					
Boc-Ala(3Cl)-OH	127–129	–	–	–	99
Boc-D-Ala(3Cl)-OH	127–129	–	–	–	99
$Cl^-H_2^+$-Ala(3Cl)-OBu^t	142–145	–	–	–	99
$Cl^-H_2^+$-D-Ala(3Cl)-OBu^t	175–180	–	–	–	99
Z-Ala(3F)-ONb	133–135	–	–	–	476
Z-Ala(3F)-OH	95–98	–	–	–	476
Fmoc-(Me)Ala-OH	154.5–155.5	−21.4	1	CH_2Cl_2	13
Arginine					
Boc-Arg(Z_2)-CHN_2	131–133	−29.5	1	DMF	190
Boc-Arg(Z_2)-CH_2Cl	97–98	−25	1.1	DMF	190
α-Aminoadipic acid (Aad)					
Z-Aad-ONp	101–104	−8.01	2	Acetone	482
Aziridine carboxylic acid (Azy)					
Boc-Azy-OH	85	−118.5	1	MeOH	7
(2*R*)-Z-Azy-OBzl	oil	+20.0	1	MeOH	540
(2*S*)-Z-Azy-OBzl	oil	−19.0	1	MeOH	540
β-Carboxyaspartic acid (Asa)					
Z-(D,L)-Asa(OBu^t)$_2$-OH	62–63	–	–	–	494
γ-Carboxyglutamic acid (Gla)					
Z-Gla(OBu^t)$_2$-OMe	72–75	−16.36	0.935	MeOH	567
Z-Gla(OBu^t)$_2$-OPcp	106–107	−16.23	1.03	EtOAc	567
$TosO^-H_2^+$-Gla(OBu^t)-OMe	95–96	+24.4	1.14	MeOH	567
Cyclohexylalanine (Cha)					
Boc-Cha-OH-DCHA	160	−8.5	1	HOAc	7
β-Cyclopropylalanine (Cpr)					
Boc-Cpr-OH-DCHA	140	+12.0	1	DMF	227

Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Boc-D-Cpr-OH-DCHA	127–131	−11.5	1	DMF	227
Tfa-D-Cpr-OH	90	–	–	–	227
Diaminobutyric acid (Dbu)					
H-Dbu(Teoc)-OH	223–224	–	–	–	29
Dipropylglycine (Dpg)					
Boc-Dpg-OH	oil	–	–	–	69
H-Dpg-OBu^t	oil	–	–	–	69
H-Dpg-OMe	b.p. 110 °C 15 mm	–	–	–	69
H-Dpg-OPh	oil	–	–	–	69
(Nps-Dpg)$_2$O	118–119	–	–	–	69
Nps-Dpg-OH	139–141	–	–	–	69
Tfa-Dpg-OH	120–121	–	–	–	69
Z-Dpg-OH	89–90	–	–	–	69
Z-Dpg-OPh	62.8–63	–	–	–	69
Glutamic acid					
Z-D-Glu–	92–93.5	+32.6	10	HOAc	460
Z-D-Glu-OH	117.5–119	+7.2	10	HOAc	460
Z-D-Glu(OH)-OBzl-DCHA	162–163.5	+11.5	2	MeOH	460
Glycine					
Boc-(4-NO$_2$-Bzl)Gly-OFm	122–123	–	–	–	19
Homoarginine					
Cl$^-$H$_2$$^+$-Har(HCl)-OMe	118–120	+34.0	1	MeOH	483
Homocysteine (Hcy)					
Boc-Hcy(Bu^t)-OH-DCHA	138–140	+29.4	1	$CHCl_3$	35
Cl$^-$H$_2$$^+$-Hcy(Bu^t)-OH	248–250	+21.6	1.1	1M HCl	35
Histidine					
Fmoc-(Me)His-Dnp-OH	160–164	−15.1	1	MeOH	13
4-Hydroxyproline (Hyp)					
Boc-Hyp-OH	120–122	−70.4	1	MeOH	7
Boc-Hyp-OH-DCHA	194–195	−23.5	1	DMF	7
Fmoc-Hyp-OH	122–124	−44.6	–	EtOAc	12
Troc-Hyp-OH-Bu^{t}NH$_2$	204–205	−46.50	5	MeOH	12
Lanthionine (Lan)					
Bis-Z-Lan bis-benzyl ester	94.5–95	−31.5	1	MeOH	540
Leucine					
Boc-4,5-ΔLeu-OH	149–150	+11.9	1	MeOH	77
Fmoc-4,5-ΔLeu-OH	130–131	−18.7	1	DMF	77
Boc-D-(Me)Leu-OH	53	+33.5	0.86	$CHCl_3$	203
D-Cl$^-$H$_2$$^+$-(Me)Leu-OBzl	124–125	−3.9 −12.2*2	0.98	$CHCl_3$	203

Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
$Cl^-H_2^+$-(Me)Leu-OBzl	123–125	+4.0 +12.0*2	0.95	$CHCl_3$	203
H-(Me)Leu-OBzl	–	−6.09	1	$CHCl_3$	203
D-H-(Me)Leu-OBzl	–	+6.06	0.9	$CHCl_3$	203
Methallylglycine (Mag)					
Boc-Mag-OBu^t	oil	−18.7	1	DMF	546
Methionine (Met)					
Boc-D-Met-OH-DCHA	138–139	−11.2	1	MeOH	7
Fmoc-(Me)Met-OH	142–146	−33.3	1	CH_2Cl_2	13
Ornithine					
$Cl^-H_2^+$-Orn(Bz)-OH	193–195	–	–	–	408
$Cl^-H_2^+$-Orn(Tos)-OH	144–145	–	–	–	408
H-Orn(HCO)-OH	208–210	+2.9	1	H_2O	261
H-Orn(Teoc)-OH	228–229.5	–	–	–	29
β-Pyrazinylalanine (Paa)					
Boc-Paa-OH	75	−16.8	1	MeOH	552
H-Paa-OH	229	−18.0	1	H_2O	552
		−23.5	1	5 M HCl	552
α-Phenylglycine (Pgl)					
Boc-D-Pgl-OH	99–101	−111.8	2.5	DMF	569
Boc-D-Pgl-OH-PEA(*S*)	156–159	−96.5	1	DMF	569
Boc-Pgl-OH-PEA(*R*)	157–160	+95.9	1	DMF	569
Ddz-Pgl-OH-DCHA	126–130	+45.54*2	1	MeOH	566
Phenylalanine					
Ac-D-Phe(NO_2)-OH	165–166	−46.2	2.8	EtOH	496
Ac-Phe(NO_2)-OMe	118–120	+15.1	2.1	EtOH	496
Ac-D-Phe(NO_2)-OMe	110–112	−13.8	2.1	EtOH	496
Z-(2*R*)-∇^EPhe-OH	153–154	+114.8	1	MeOH	570
Z-(2*RS*)-∇^2Phe-OH	168–169	–	–	–	570
Z-(2*S*)-∇^EPhe-OH	153–154	−120.1	1.03	MeOH	570
Boc-Phe(4Cl)-OH	104	+14.4	1	MeOH	7
Boc-Phe(4NO_2)-OEt	61	−20.0	1	MeOH	483
Boc-Phe(4NO_2)-OH	200	+8.5	1	MeOH	7
$Cl^-H_2^+$-Phe(NO_2)-OMe	218–219	+11.5	0.9	H_2O	496
Fmoc-D-Phe-OH	176–181.5	+37.8	0.87	DMF	91
H-Phe(4SO_3H)-OH	–	−11.8	–	–	176
Boc-(Me)Phe-OH	85–86	−84.9*2	–	MeOH	566
Boc-Phe-ol	91–93	−29.6	1	EtOH	45
Pipecolinic acid (Pip)					
Boc-Pip-OH	110	−35.3	1	MeOH	7

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Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
$Cl^-H_2^+$-Pip-OMe	169	−7.47*2	0.5	MeOH	566
Fmoc-Pip-OH	147–150	−24.0	1	MeOH	56
Fmoc-Pip-OPfp	95–96	−45.8	1	$CHCl_3$	56
Z-Pip-OH	102–105	−37.95*2	1	EtOH	566
Propargylglycine (Prg)					
Ac-D,L-Prg-OH	137–139	–	–	–	99
Boc-Prg-OH	95–97	–	–	–	99
$Cl^-H_2^+$-Prg-OH	195–197	–	–	–	99
$Cl^-H_2^+$-Prg-OH	212–214	+8.1	1	5 M HCl	99
Proline					
Boc-D-Pro-OH	130–134	+60.1	1	MeOH	7
Boc-D-Pro-OH-DCHA	133–135	+61.0	1	HOAc	7
Boc-D,L-ΔPro-OH	100	–	–	–	7
Boc-3,4-ΔPro-OH	86–87	−264	1.36	MeOH	77
D,L-*cis*-Pro(5Et)	145–147	–	–	–	372
D,L-*trans*-$Cl^-H_2^+$-Pro(5Et)	119–120	–	–	–	372
Serine					
Fmoc-(Me)Ser(Bzl)-OH	oil	−6.4	1	CH_2Cl_2	13
Fmoc-(Me)Ser(Bzl)-OMe	oil	−10.3	1	CH_2Cl_2	13
Threonine					
Boc-D-*allo*-Thr-OH-DCHA	155	−10.7	1	MeOH	7
Z-(D,L)-*allo*-Thr-OBzl	67–68	–	–	–	465
Tryptophan					
Fmoc-D-Trp-OH	165–166.5	+26.8	1.08	DMF	91
Tyrosine					
Boc-D-Tyr-OH-DCHA	201–202	−27.6	1	MeOH	7
H-Tyr(PO_3H_2)-OH	226–227	−7.8	1	2 M HCl	571
Valine					
Boc-D-(Me)Val-OH	88	+58.0	1	$CHCl_3$	203
$Cl^-H_2^+$-(Me)Val-OBzl	151–153	+1.0*2	1.05	$CHCl_3$	203
D-$Cl^-H_2^+$-(Me)Val-OBzl	150–153	−1.0*2	1	$CHCl_3$	203
Fmoc-(Me)Val-OH	185–187	−31.2	1	CH_2Cl_2	13
H-(Me)Val-OBzl	–	−5.2	1.18	$CHCl_3$	203
D-H-(Me)Val-OBzl	–	+5.1	1	$CHCl_3$	203

6 Appendix III: Purification Methods

Methods for the purification of protected peptides and proteins are given; the list also includes references to the purification of free peptides and separation of diastereoisomers.

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Technique	*Ref.*
High-performance Liquid Chromatography	
H.p.l.c. separation of ACTH fragments	572
H.p.l.c. on a new crosslinked agarose	135
H.p.l.c. separation of angiotensin I and II diastereoisomers	573
Separation of α- and β-aspartyl peptides by h.p.l.c.	133
H.p.l.c. of carbodi-imides	574
H.p.l.c. of cytochrome C peptides	575
Separation of diketopiperazine diastereoisomers by h.p.l.c.	576
H.p.l.c. of dipeptides	577
H.p.l.c. of Dns-amino acids	578, 579
H.p.l.c. of Dopa enantiomers	580
H.p.l.c. enantiomer separation	581, 582, 583, 584, 585
H.p.l.c. of enkephalin and β-endorphin	586
H.p.l.c. (gel permeation)	587, 588
H.p.l.c. of glucagon	589
H.p.l.c. of haemoglobin variants	590
H.p.l.c. of four mono-iodinated insulins	591
Separation of myelin basic protein (43–88) by h.p.l.c.	592
Oligopeptide mobility on h.p.l.c. (porasil column)	593
H.p.l.c. separation of oxytocin analogues	594

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Peptide	*Ref.*
H.p.l.c. of parathyroid hormone (7–34)	595
H.p.l.c. of peptides	596
Separation of phenylthiohydantoins by h.p.l.c.	597, 598
Purification of porcine pro-insulin by h.p.l.c.	599
H.p.l.c. separation of phosphorylamino acids	600
Analysis of 5-pyrrolidone-2-carboxylate esters by h.p.l.c.	601
Retention times of histidine peptides in reversed-phase h.p.l.c.	134
Reversed-phase h.p.l.c. of peptides	602
Reversed-phase and ion-exchange h.p.l.c.	603
H.p.l.c. determination of secondary amines	604
H.p.l.c. on Sephadex LH60 and Spheron P40	605
H.p.l.c. of tryptophan-containing peptides	606
H.p.l.c. of tyrosine peptides	607
H.p.l.c. of secretin	589
Gas–Liquid Chromatography	
Amino acid enantiomer separation on glass capillary columns using derivatives of XE60	608
G.l.c. separation of amino acid enantiomers	609
G.c. of acylamino acid esters	610
G.c. of *N*(*O*,*S*)-Tfa-amino acid n-propyl esters	611, 612
Other Chromatographic Methods	
Dipeptidyl arginal affinity resin	613
Ion exchange on TSK-gel (CM-Toyopearl 650)	614
Ligand-exchange liquid chromatography	615

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Peptide	*Ref.*
Liquid chromatography of amino acids and thiols	616
Separation of enantiomers by ligand-exchange liquid chromatography	617
Resolution of amino acid enantiomers on a modified chiral silica-gel column	618
Reversed-phase l.c., retention times	619
Purification of phosphopeptides	620
H.p.t.l.c. separation of di- and tri-peptide diastereomers	621
Separation of β-endorphin, β-lipotropin, and enkephalins by reversed-phase t.l.c.	622
T.l.c. of amino acid derivatives on silanized silica gel	623

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4

Peptides with Structural Features not Typical of Proteins

BY P. M. HARDY

1 Introduction

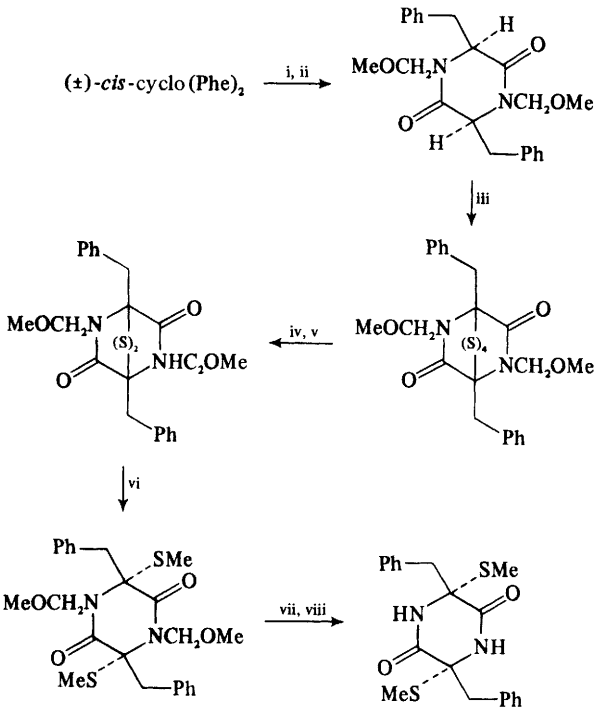
The number of references in the area covered by this 1983 chapter has increased by nearly a quarter compared to those of 1982. This has necessitated some use of tables, in particular in the section on linear peptides, in order to keep to the allotted number of pages. Otherwise, the type and scope of coverage remain essentially as in earlier volumes, although the subdivisions of the section on linear peptides have been redefined to accommodate the increasing amount of synthetic work falling in this category.

2 Cyclic Peptides

2,5-Dioxopiperazines (Cyclic Dipeptides). — The first naturally occurring sulphur-containing 2,5-dioxopiperazine unsubstituted on the nitrogen (1) has been isolated from *Aspergillus terreus*. This is thought to be a biosynthetic precursor of bisdethiobis(methylthio)acetylaranotin, and it has been synthesized (Scheme 1).[1] Cyclo(His-Pro) has now been found in rat pancreatic islets, and it may play a role in modulating pancreatic responses to nutrient and pharmacological stimuli.[2] The incorporation of (2*RS*)-[indole-2-^{13}C, 2-^{15}N]tryptophan into both roquefortine (2) and oxaline (3) and the efficient conversion of roquefortine into oxaline define a biosynthetic pathway (Scheme 2) by which N-14 of oxaline is derived from N-5 of roquefortine, and thus from tryptophan.[3]

The preparation of 2,5-dioxopiperazines by refluxing methanolic solutions of dipeptide methyl esters is claimed to give better yields and less racemization than the Fischer or Nitecki methods. Most of the racemization that does occur was found to take place after cyclization.[4] A study in aqueous solution in the neutral pH region of Leu-Gly-Gly and Gly-Leu-Gly at 130 °C and of Phe-Gly-Leu-Gly-Val-Gly at 100 °C shows that cyclic dipeptides are split off from the

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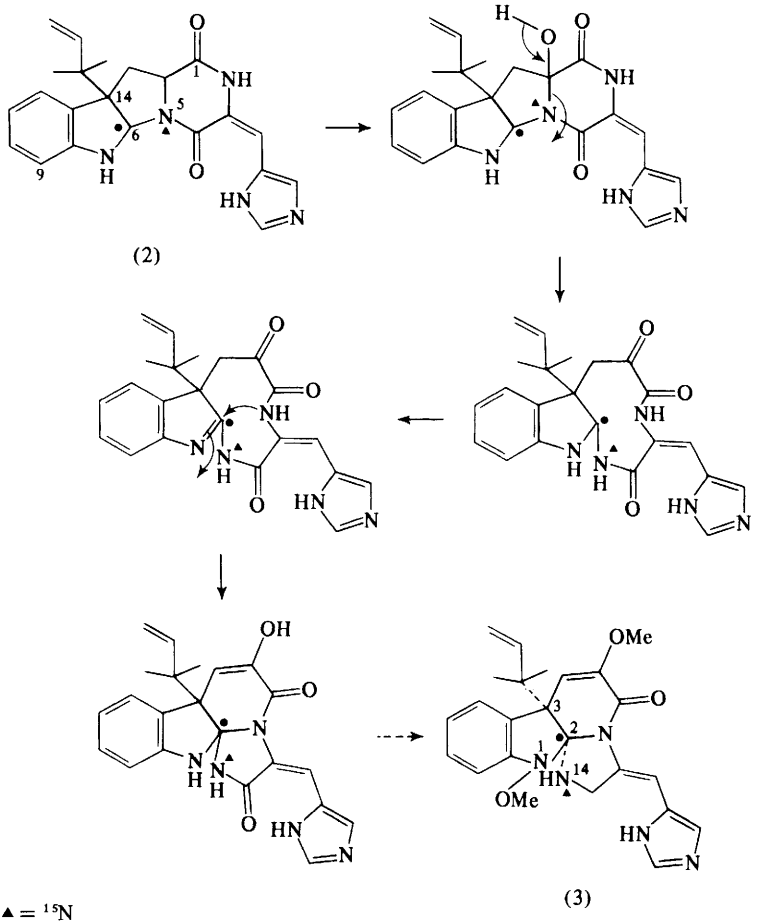
(1)

Reagents: i, $KOBu^t$; ii, $MeOCH_2Cl$–DMSO; iii, LDA, S, THF–DMF, −78 °C; iv, $NaBH_4$, THF–EtOH, 0 °C; v, EtOH, $FeCl_3$; vi, MeI; vii, BBr_3; viii, $NaHCO_3$

Scheme 1

N-terminal region of the peptides.[5] A series of 30 2,5-dioxopiperazines has been synthesized as analogues of cyclo(Tyr-Arg), and their analgesic activities have been evaluated after intracerebral administration in mice. By far the most potent compound prepared was cyclo[Tyr(Et)-Har] (Har = homoarginine), which proved nearly as potent as morphine on a molar basis. Further extension of the *O*-alkyl side chain tended to decrease activity.[6] Four cyclic (*Z*)-dehydrodipeptides (4) have been prepared from cyclo(*N*-AcGly-*N*-AcAla) by condensation with the corresponding aldehydes. After hydrazinolytic removal of the acetyl groups, chiral induction on hydrogenation (Pd, DMF) was evaluated. Both low temperatures (0 °C) and increasing chain length gave the best results. A planar orientation of the heterocyclic and aromatic rings appears to be important in inducing high asymmetry.[7]

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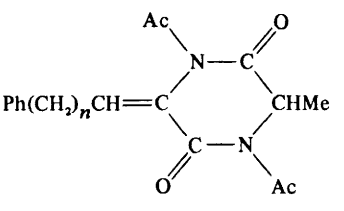


(2)

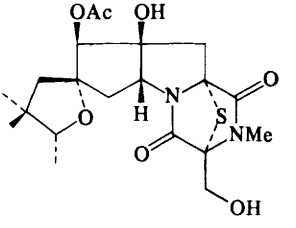
(3)

● ^{13}C, ▲ = ^{15}N

Scheme 2



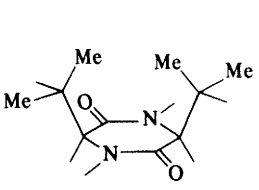
(4) n = 0, 1, 2, or 3



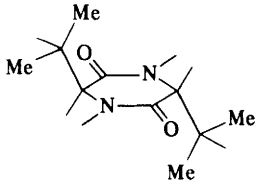
(5)

On treatment with triphenylphosphine, the epidithiopiperazinedione sirodesmin is converted into the corresponding monosulphide (5); *X*-ray analysis showed retention of configuration at both bridgehead carbon atoms, a contrast

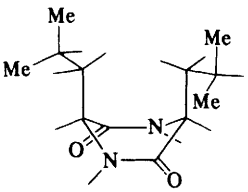
to earlier work on gliotoxin.[8] An *X*-ray examination of the Ag^I complex of cyclo(Met)$_2$ shows that each Ag atom is co-ordinated linearly to two S atoms to give a helical polymer along the *b*-axis,[9] while the crystal of cyclo(Met)$_2$ shows quasi-equatorial carbamoylmethyl substituents and no intramolecular H bonds, the packing being dominated by intermolecular H bonds.[10] The most probable solution conformation of four cyclic dipeptides, cyclo(Val)$_2$ (6), cyclo(Val-D-Val) (7), cyclo(Leu)$_2$ (8), and cyclo(Leu-D-Leu) (9), has been suggested on the basis of ^{1}H n.m.r. and c.d. spectra. It is thought that the *cis* cyclic dipeptides of this type adopt the conformation shown because of hydrophobic interactions between the side chains.[11]



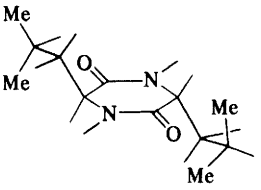
(6)



(7)



(8)



(9)

The total synthesis of bicyclomycin (10) from 2,5-dioxopiperazine in 19 steps has been reported. The second half of the synthesis is shown in Scheme 3. Addition of methylglyceraldehyde acetonide gave compound (11) as a mixture of four stereoisomers with the major component the desired product; this was separated by preparative t.l.c.[12] Other studies related to bicyclomycin syntheses have also appeared. Regioselective functionalization of bicyclic piperazinedione bridgehead carbanions can be achieved (Scheme 4),[13] and a 3-acetoxypiperazine-2,5-dione derivative (12) has been prepared as a structural prototype for the

[8] J. P. Férézou, A. Quesneau-Thierry, M. Césario, C. Pascard, and M. Barbier, *J. Am. Chem. Soc.*, 1983, **105**, 5402.

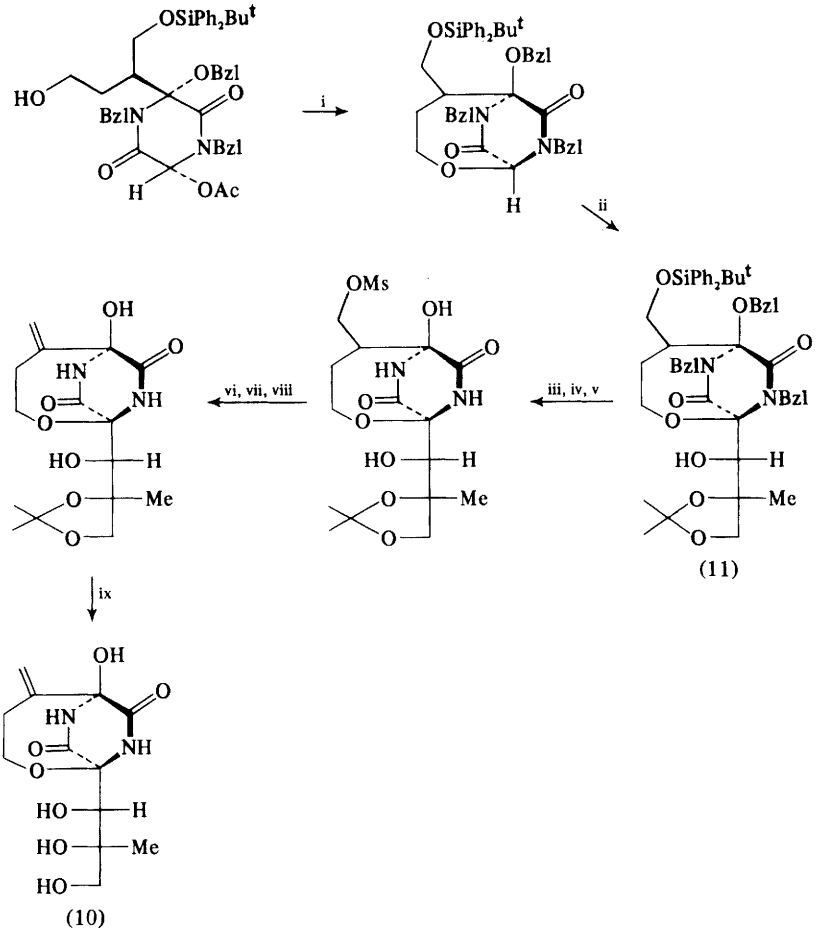
[9] Y. Kojima, T. Yamashita, Y. Ishino, T. Hiroshima, and K. Hiratsu, *Chem. Lett.*, 1983, 453.

[10] C. Howes, N. W. Alcock, B. T. Golding, and R. W. McCabe, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2287.

[11] M. Tanihara, T. Hiza, Y. Imanishi, and T. Higashimura, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1155.

[12] S. Nakatsuka, K. Yamada, K. Yoshida, O. Asano, Y. Murakami, and T. Goto, *Tetrahedron Lett.*, 1983, **24**, 5627.

[13] R. M. Williams, J. S. Dung, J. Josey, R. W. Armstrong, and H. Meyers, *J. Am. Chem. Soc.*, 1983, **105**, 3214.

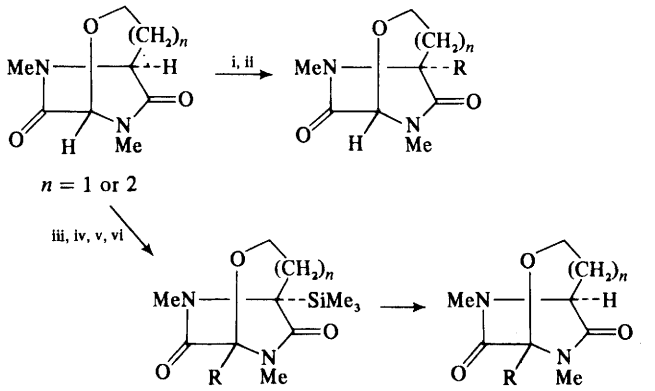


Reagents: i, Py–HOTs; ii, Bu^nLi, (±)-2-methylglyceraldehyde acetamide; iii, $Bu^n{}_4NF$–THF; iv, H_2, Pd/C; v, MsCl–py; vi, $Na[PhSeBH_3]$; vii, *m*-CPBA; viii, 60 °C; ix, 0.2N H_2SO_4

Scheme 3

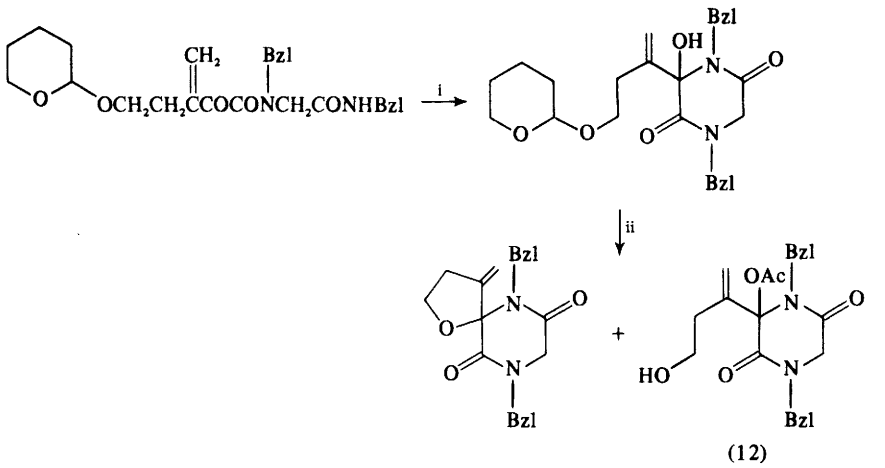
study of ring closure to bicyclomycin using an acyclic compound until a relatively late stage in the synthesis (Scheme 5).[14] Acid degradation of bicyclomycin gives dispiropiperazinedione derivatives, and a new route to such compounds has been developed (Scheme 6).[15] 7-*N*-Methyl-3′-*O*-benzoyl bicyclomycin (13) has been found to rearrange on attempted methylation (Scheme 7). This rearrangement was also found to occur under very mild conditions; when compound (13) is stored in DMSO at 25 °C, formation of compound (14) is almost complete

[14] P. Yates and J. H. Hoare, *Can. J. Chem.*, 1983, **61**, 519, 1397.
[15] C. Shin, Y. Sato, S. Honda, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2652.



Reagents: i, LDA–HMPA; ii, RI; iii, LDA, THF, HMPA; iv, Me_3SiCl; v, LDA; vi, RI; vii, Bu_4NF, THF

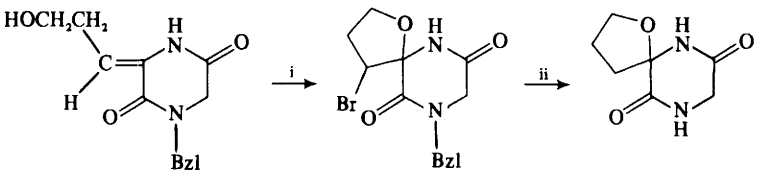
Scheme 4



(12)

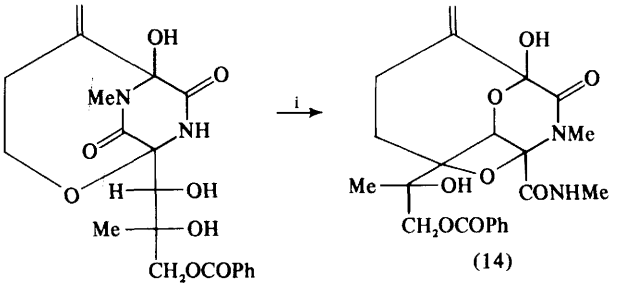
Reagents: i, Mg Pr^{i}cyclohexylamide; ii, HAc, H_2O, THF

Scheme 5



Reagents: i, NBS, $CHCl_3$; ii, Pd/C, H_2, NEt_3

Scheme 6



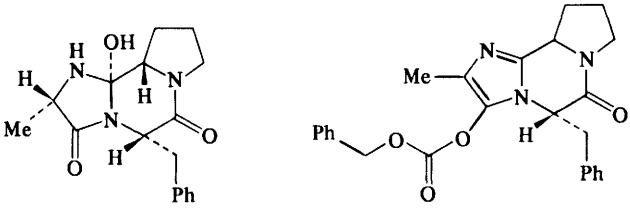
(13) (14)

Reagent: i, MeI, K_2CO_3, DMF

Scheme 7

after 96 h. Although 7,9-*N*,*N*-dimethylbicyclomycin also rearranges similarly, 7-*N*-monomethylbicyclomycin is resistant to this reaction.[16]

N-Alkylated 2,5-dioxopiperazines bridged between the 3- and 6-positions by two or three methylene groups can be lithiated at the bridgehead positions and then deuteriated or alkylated, showing that the attachment of sulphur substituents to the bridgeheads is not a necessary requirement for lithiation to occur.[17] *N*-(*p*-Methoxybenzyl) groups in some dioxopiperazine derivatives have proved to be efficiently removed with ceric ammonium nitrate in aqueous acetonitrile without affecting *N*-benzyl, methoxyl in the aminal structure, and isolated vinyl groups.[18] A free azacyclol (15) has been prepared by direct cyclization of Ala-Phe-Pro-ONp in mildly alkaline aqueous medium or by hydrogenolysis of its *N*-benzyloxycarbonyl derivative. It proved stable enough to be stored for several weeks at ambient temperature. Carbobenzoxylation of compound (15) lead to compound (16), indicating that dehydration precedes *O*-acylation.[19]



(15) (16)

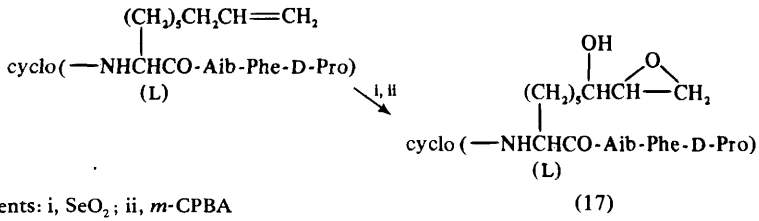
The enantioselective synthesis of non-proteinogenic amino acids *via* the metallated bis-lactim ethers of cyclic dipeptides has been reviewed.[20]

Larger Cyclic Peptides. — The cyclic tripeptide cyclo(Pro_2NBGly) (NB = *o*-nitrobenzyl) has been prepared. In the crystal it adopts a boat conformation of

[16] O. Wacker, W. Kump, and B. W. Muller, *Tetrahedron Lett.*, 1983, **24**, 5607.
[17] F. W. Eastwood, D. Gunawardana, and G. T. Wernert, *Aust. J. Chem.*, 1982, **35**, 2289.
[18] Y. Yoshimura, M. Yamaura, T. Suzuki, and H. Hashimoto, *Chem. Lett.*, 1983, 1001.
[19] G. Lucente, F. Pinnen, A. Romeo, and G. Zanotti, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1127.
[20] U. Schollkopf, *Tetrahedron*, 1983, **39**, 2085.

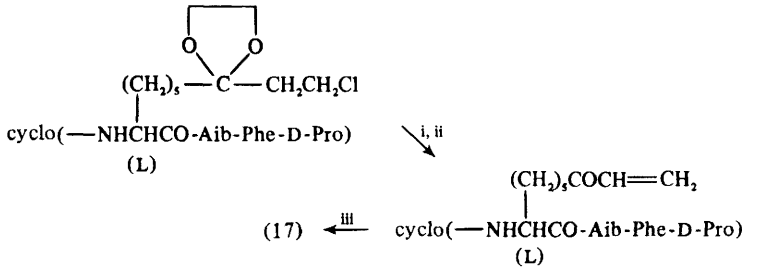
the ring backbone, but in solution there is a solvent-dependent equilibrium, slow on the n.m.r. time-scale, between a boat and crown conformation.[21] By contrast, a ^{1}H and ^{13}C n.m.r. study of cyclo(Pro-BzlGly-D-Pro) indicates a rapid equilibrium between two boat conformations. The one that predominates (80%) is also found in the *X*-ray crystal structure.[22]

Two total syntheses of the cytostatic cyclic tetrapeptide chlamydocin (17) have been reported. In one (Scheme 8), the side chain of the 2-amino-8-oxo-9,10-epoxydecanoyl (Aoe) residue was elaborated after cyclization from a terminal alkene group by successive allylic oxidation, epoxidation, and oxidation of the resulting secondary alcohol to a ketone. The product was assumed to be a mixture of (9*RS*)-Aoe diastereoisomers, having half the inhibitory activity of natural chlamydocin with respect to the uptake of [^{3}H]thymidine in PHA-induced bovine lymphocytes. This suggests that the (9*R*) isomer is much less potent than the natural (9*S*) compound.[23] In the other synthesis cyclization again preceded adjustment of functionality in the Aoe side chain (Scheme 9), leading to the same diastereoisomers.[24] A spectroscopic investigation of the conformations of chlamydocin and [Ala^{4}]-chlamydocin in $CHCl_3$ indicates that both compounds adopt the all-*transoid* bis-γ-turn conformation previously shown by *X*-ray to



Reagents: i, SeO_2; ii, *m*-CPBA

Scheme 8



Reagents: i, oxalic acid, dioxan, H_2O; ii, NaAc; iii, Bu^{t}OOH, $KHCO_3$

Scheme 9

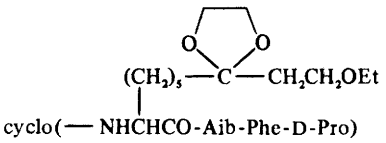
[21] H. Kessler, R. Schuck, R. Siegmeier, J. W. Bats, H. Fuess, and H. Forster, *Liebigs Ann. Chem.*, 1983, 231.

[22] H. Kessler, W. Bermel, G. Krack, J. W. Bats, H. Fuess, and W. E. Hull, *Chem. Ber.*, 1983, **116**, 3164.

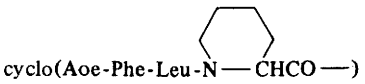
[23] M. Kawai and D. M. Rich, *Tetrahedron Lett.*, 1983, **24**, 5309.

[24] U. Schmidt, T. Beuttler, A. Lieberknecht, and H. Griesser, *Tetrahedron Lett.*, 1983, **24**, 3573.

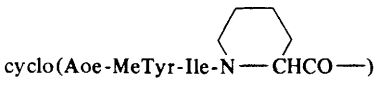
exist in the crystals of dihydrochlamydocin.[25] Addition of >50% DMSO, however, gives a mixture of this conformer and the *cis,trans,trans,trans* form, hitherto unobserved in cyclic tetrapeptides.[26] A two-dimensional n.m.r. study of a compound (18) closely related to chlamydocin shows the bis γ-turn conformation in $CHCl_3$ and two conformations in DMSO that slowly (n.m.r. time-scale) interconvert.[27] It would seem likely that these forms are those delineated above for chlamydocin itself. An anti-tumour antibiotic WF-3161 from the fungus *Petriella guttulata* has a cyclic tetrapeptide structure (19) differing in three amino acid residues from chlamydocin and HC-toxin.[28] It is more closely related to Cyl-2 (20).



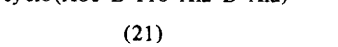
(18)



(19)



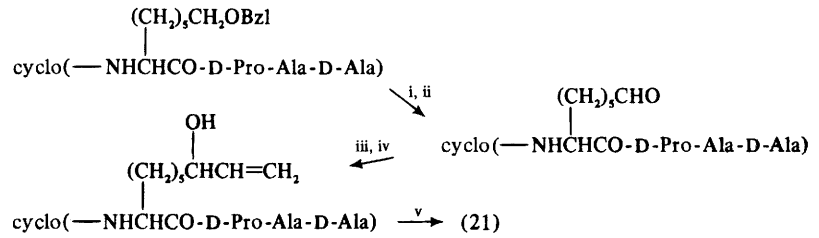
(20)



(21)

Four groups now concur in the revision of the structure of HC-toxin to compound (21), with a D-alanine residue replacing its enantiomer.[29,30,31,32] N.m.r. studies by two of these groups indicate that in solution HC-toxin adopts the bis γ-turn conformation found previously for chlamydocin, all amide bonds being *trans*.[31,32] Final confirmation of the structure of HC-toxin comes from its synthesis, the Aoe side chain again being generated at the end of the synthesis (Scheme 10). The synthetic material (a mixture of the two epoxide epimers) had an ED_{50} of 0.85 μg ml^{-1} compared to 0.5 μg ml^{-1} for natural HC-toxin in the modified root-growth assay against susceptible and resistant maize hybrids.[33] A lactam analogue of the cyclic tetradepsipeptide AM-toxin 1, [Val^2,$Tyr(Me)^3$]-AM-toxin 1, has been prepared. Its minimum toxic concentration for necrotic

[25] D. M. Rich, M. Kawai, and R. D. Jasensky, *Int. J. Peptide Protein Res.*, 1983, **21**, 35.
[26] M. Kawai, R. D. Jasensky, and D. M. Rich, *J. Am. Chem. Soc.*, 1983, **105**, 4456.
[27] E. Hashinger and H. Kalchauser, *Tetrahedron Lett.*, 1983, **24**, 2553.
[28] K. Umehara, K. Nakahara, S. Kiyoto, M. Iwami, M. Okamoto, H. Tanaka, M. Kohsaka, M. Aoki, and M. Imanaka, *J. Antibiot.*, 1983, **36**, 468.
[29] J. D. Walton, E. D. Earle, and B. W. Gibson, *Biochem. Biophys. Res. Commun.*, 1982, **107**, 785.
[30] M. R. Pope, L. M. Ciuffetti, H. W. Knoche, D. McCrery, J. M. Daly, and L. D. Dunkle, *Biochemistry*, 1983, **22**, 3502.
[31] M. Kawai, D. M. Rich, and J. D. Walton, *Biochem. Biophys. Res. Commun.*, 1983, **111**, 398.
[32] P. Mascagni, M. Pope, and W. A. Gibbons, *Biochem. Biophys. Res. Commun.*, 1983, **113**, 10.
[33] D. M. Rich and J. H. Gardner, *Tetrahedron Lett.*, 1983, **24**, 5305.

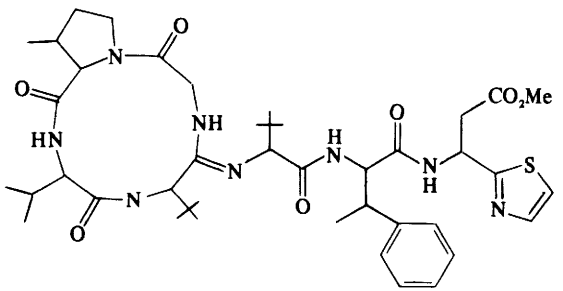


Reagents: i, Pd/C, H_2; ii, py-SO_3, DMSO, NEt_3; iii, LiC≡CH; iv, H_2, Lindlar catalyst; v, *m*-CPBA

Scheme 10

activity on apple leaf was 0.1 μg ml^{-1} compared to 0.002 μg ml^{-1} for AM-toxin 1 itself.[34]

Bottromycin A2 can be regarded as a cyclic tetrapeptide with one of the amide bonds replaced by an amidine group. On the basis of its ^{15}N and ^{1}H n.m.r. spectra, its structure has been revised to that of compound (22), the *N*-substituent on the peptide cycle now being on the other nitrogen atom of the amidine moiety.[35] In solution, n.m.r. evidence indicates that cyclo(Pro-Sar)$_2$ exists as an equilibrium mixture of a C_2 symmetric form and an asymmetric form, the latter being the minor component in polar solvents. This is the first example of a cyclic tetrapeptide conformation containing all four peptide bonds in the *cis* mode;[36] its existence is confirmed by *X*-ray analysis of the crystalline peptide.[37] In solution cyclo[Gly-Cys(BzlOMe)-Sar-Pro] appears to equilibrate between three conformers, a γ-turn being present in two of them. This cyclic tetrapeptide forms a 1:1 complex with both Li^{I} and Mg^{II}, but with Ca^{II} and Ba^{II} it forms at least three types of complex with each cation.[38] Cyclo(Gly-Cys-Sar-Pro) has been prepared and oxidized to a bis(cyclic tetrapeptide). Before oxidation the ring

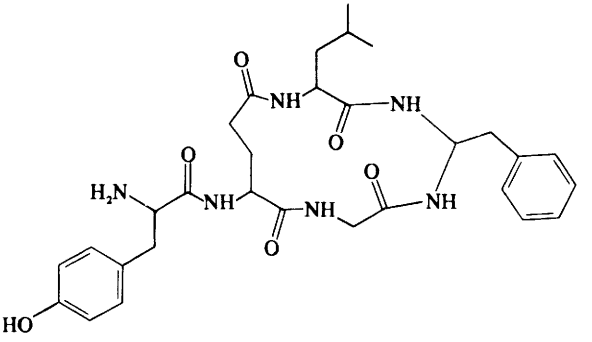


(22)

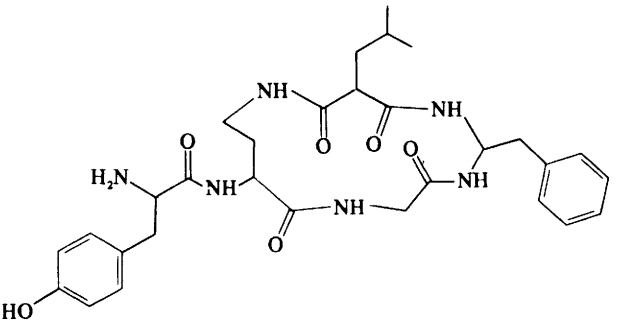
[34] T. Kozono, T. Kanmera, T. Kato, T. Ueno, and N. Izumiya, *Agric. Biol. Chem.*, 1983, **47**, 2631.
[35] D. Schipper, *J. Antibiot.*, 1983, **36**, 1076.
[36] T. Shimizu, Y. Tanaka, and K. Tsuda, *Biopolymers*, 1983, **22**, 617.
[37] K. Ueno and T. Shimizu, *Biopolymers*, 1983, **22**, 633.
[38] T. Shimizu, Y. Tanaka, and K. Tsuda, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 3817.

exists in DMSO as a major conformer equilibrating with two minor ones. After oxidation spectral studies indicate an overall 'castanet'-type structure for the molecule, the peptide rings not being parallel with the S—S bond, adopting *P*-chirality with $\theta = 90\text{–}100°$.[39]

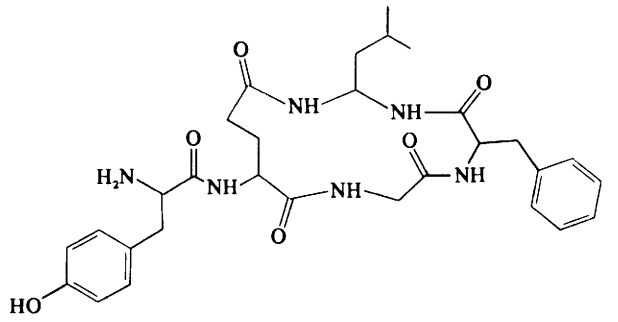
Three cyclic partial *retro-inverso* modified enkephalins (23), (24), and (25) have been prepared. Relative to Leu-enkephalin, the modified compounds are



(23)



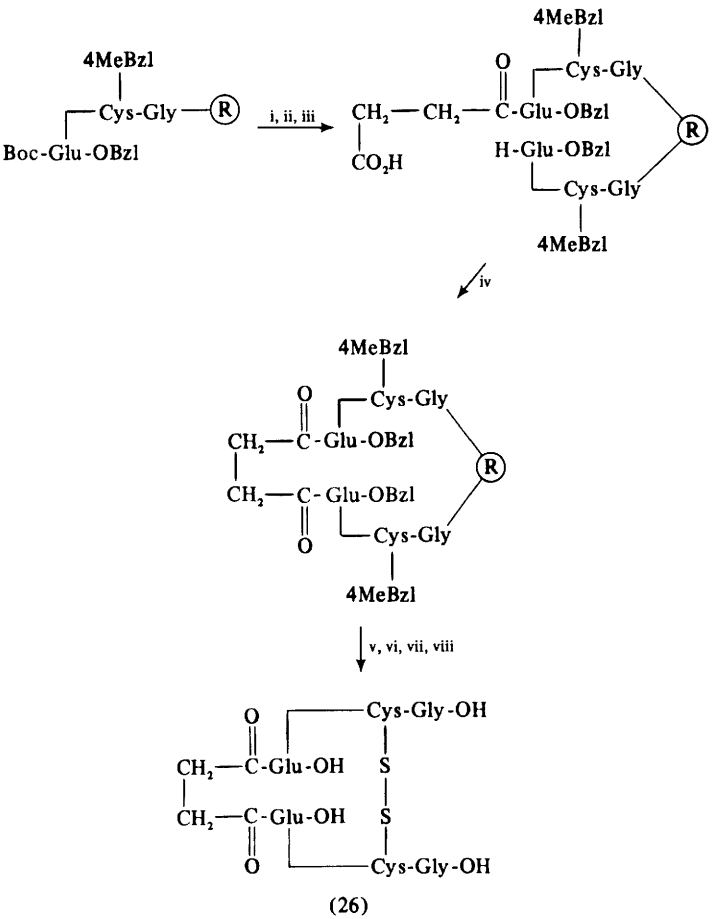
(24)



(25)

[39] T. Shimizu, Y. Tanaka, and K. Tsuda, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 3808.

all more potent in the guinea-pig-ileum assay and more effectively displace [^{3}H]naloxone from rat-brain receptors, but they are less potent in the mouse vas deferens assay and only weakly able to displace [^{3}H](D-Ala^2,D-Leu^5)-enkephalin.[40] A cyclic 'tetrapeptide' analogue of oxidized glutathione (26) has been synthesized (Scheme 11) as a probe to examine the mechanism of action of glutathione reductase. Success in this synthesis depended on the achievement of a high yield of intersite reaction within the same resin bead (less than 0.5% of the chains remained non-crosslinked).[41]

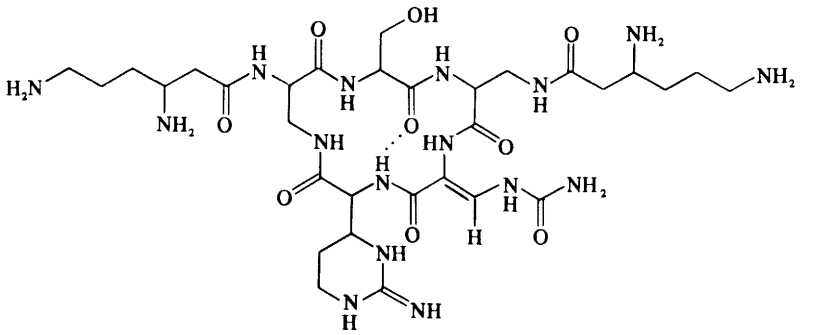


Reagents: i, TFA; ii, DIEA; iii, 0.5 mole succinic anhydride; iv, DCCI, HOBt; v, HF; vi, 10% HOAc; vii, DTT, pH 8.4; viii, O_2

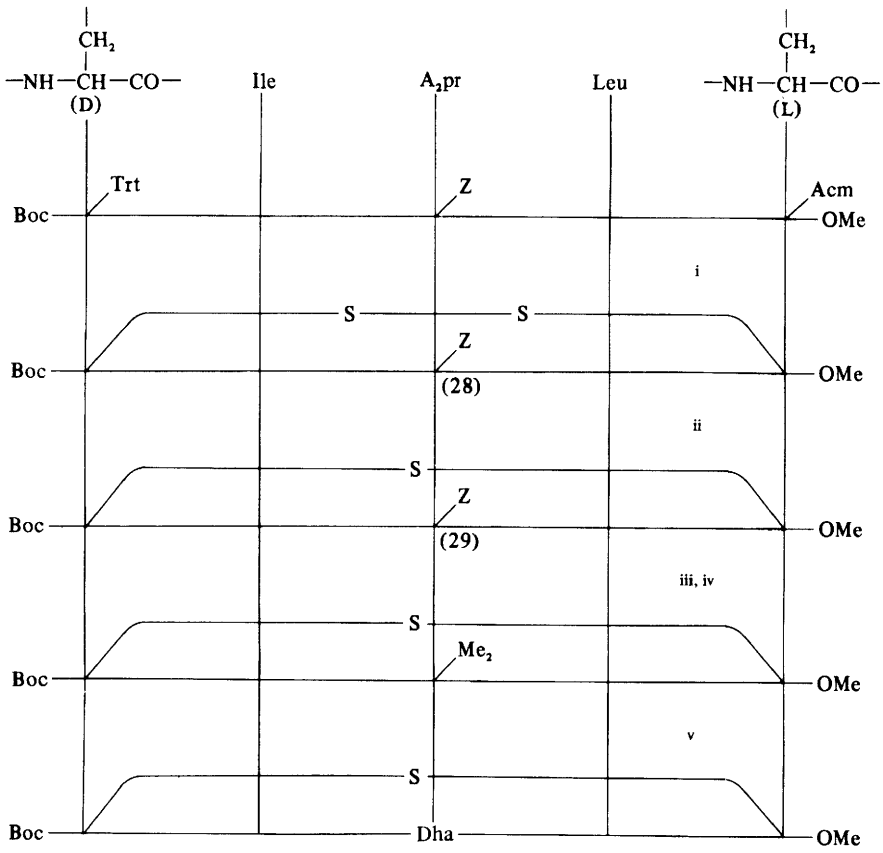
Scheme 11

[40] J. M. Berman, M. Goodman, T. M.-D. Nguyen, and P. W. Schiller, *Biochem. Biophys. Res. Commun.*, 1983, **115**, 864.

[41] K. K. Bhargava, V. K. Sarin, N. Le Trang, A. Cerami, and R. B. Merrifield, *J. Am. Chem. Soc.*, 1983, **105**, 3247.



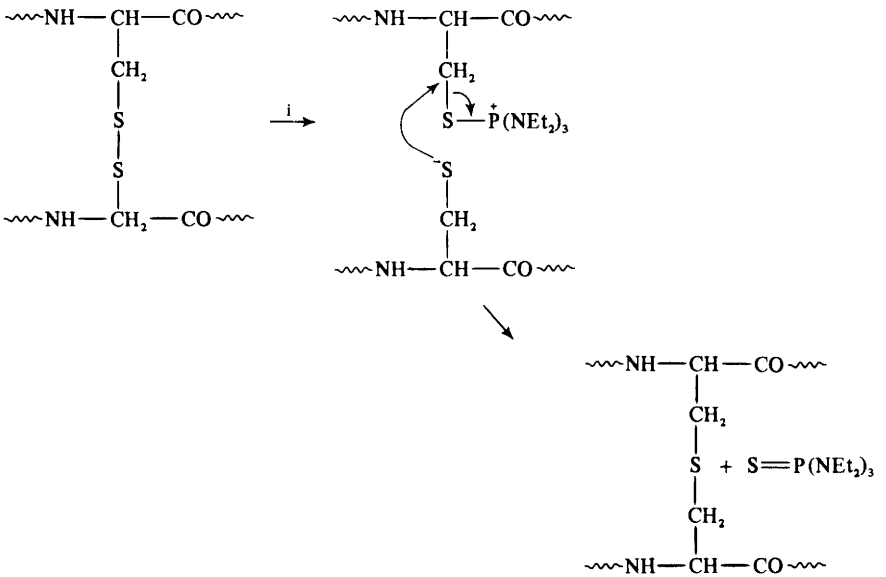
(27)



Reagents: i, I_2, MeOH; ii, $P(Et_2N)_3$; iii, H_2, Pd; iv, CH_2O, $NaBH_3CN$; v, MeI, K_2CO_3 (Hofmann degradation)

Scheme 12

Moving on to cyclic pentapeptides, the semisynthesis has been reported of di-β-lysylcapreomycin IIa (27), a capreomycin analogue effective against viomycin-resistant mutants of *Mycobacterium smegmatis*.[42] Ring A of nisin has also been prepared for the first time (Scheme 12), the 2,3-diaminopropionyl (A_2pr) residue functioning as a precursor for dehydroalanine. The partial desulphurization of the disulphide (28) was effected with tris(diethylamino)phosphine, thought to act as in Scheme 13. Removal of the *N*-benzyloxycarbonyl group from compound (29), even in the presence of sulphur, was effected in MeOH–AcOH if a relatively large amount of catalyst was used.[43] A study of the cyclization of Gly-Ala-D-Val-Leu-Ile has been made (Table 1), the insolubility of the



Reagents: i, $P(NEt_2)_3$

Scheme 13

Table 1

Method	*% Yield*	*Racemization*
Azide	53	Little
ONp	57	Extensive
NDPP*	18	Little

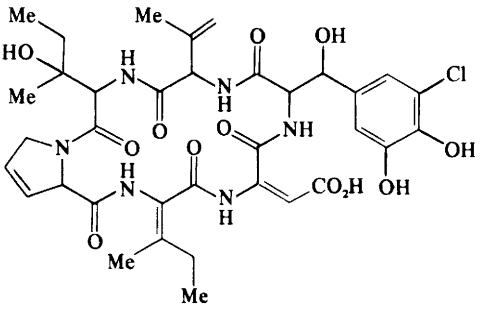
* NDPP = 5-norbornene-2,5-dicarboxamido-diphenyl phosphate

[42] T. Wakamiya and T. Shiba, *J. Antibiot.*, 1983, **36**, 197.
[43] T. Wakamiya, K. Shimbo, A. Sano, K. Fukase, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2044.

cyclic product facilitating its isolation, and the C-terminal isoleucine permitting a check on racemization, using an amino acid analyser to quantitate any *allo*-isoleucine formed. Methods based on the free linear pentapeptide and condensing agents gave only trace amounts of cyclic material, so it was concluded that it is advantageous, in this case at least, to separate the steps of activation and peptide-bond formation.[44]

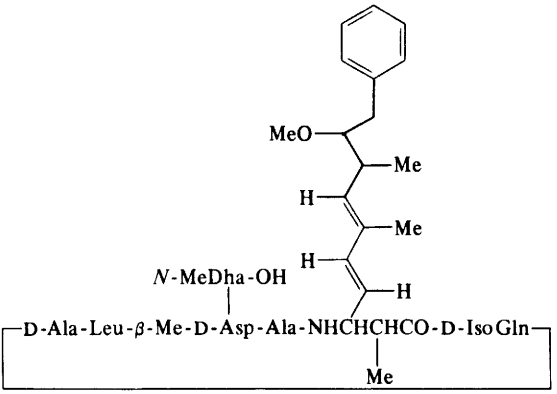
Cyclo(Pro-Phe-Gly-Ser-Gly) and cyclo(Pro-Ser-Gly-Phe-Gly) have been prepared in 42% and 30% yields, respectively, by azide cyclizations. Two-dimensional n.m.r. in DMSO shows both to adopt a βIγ^{i} conformation, but differences exist in their conformational stability.[45] A modification of cyclo(Gly-Pro-Gly-D-Phe-Pro) in which the Pro-2—Gly-3 amide bond is replaced by $-CH_2S-$ has been made, this being the only amide bond not thought to be involved in intramolecular H bonding. Analysis of the n.m.r. spectrum of this analogue in $CHCl_3$ indicates the retention of the β- and γ-turns seen in the parent, but on addition of DMSO a more flexible conformation involving a *cis–trans* proline isomerism appears.[46]

A number of novel naturally occurring cyclic hexapeptides have been reported in 1983. Phomopsin A, the main mycotoxin of cultures of *Phomopsis leptostramiformis* and the cause of lupinosis disease in animals grazing on infected lupins, is a cyclic hexapeptide containing three unsaturated amino acids. The sequence (30) is based on heteronuclear ^{13}C-$\{^{1}H\}$ selective-population inversion n.m.r. experiments.[47] Other animal poisoning in many parts of the world has been attributed to sporadic blooms of toxic strains of the blue-green algae *Microcystis aeruginosa*. The primary structure of one component, toxin variant BE-4 (31), has been determined and shown to contain a novel β-amino acid.[48] A number of closely related bicyclic hexapeptides (32) with anti-tumour activity

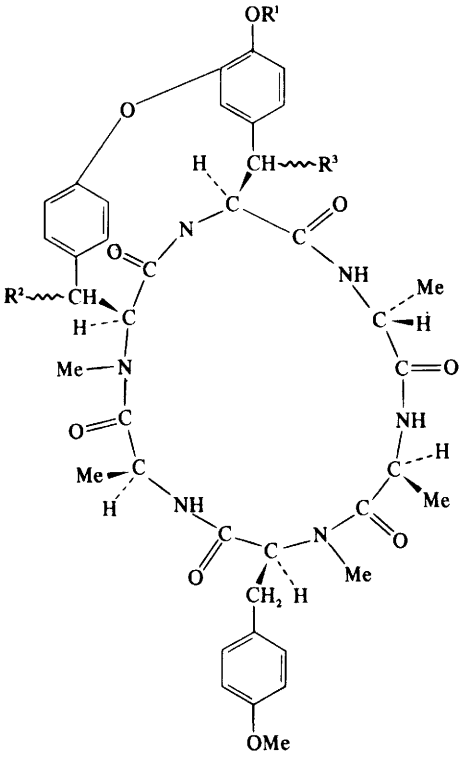


(30)

[44] A.-X. Ji and M. Bodanszky, *Int. J. Pept. Protein Res.*, 1983, **22**, 590.
[45] H. Kessler and H. Kogler, *Liebigs Ann. Chem.*, 1983, 316.
[46] A. F. Spatola, L. M. Gierasch, and A. L. Rockwell, *Biopolymers*, 1983, **22**, 147.
[47] C. C. J. Culvenor, P. A. Cockrum, J. A. Edgar, J. L. Frahn, C. P. Gorst-Allman, A. J. Jones, W. F. O. Marasas, K. E. Murray, L. W. Smith, P. S. Steyn, R. Vleggar, and P. L. Wessels, *J. Chem. Soc., Chem. Commun.*, 1983, 1259.
[48] S. Santkarn, D. H. Williams, R. J. Smith, S. J. Hammond, D. P. Botes, A. Tuinman, P. L. Wessels, C. C. Viljoen, and H. Kruger, *J. Chem. Soc., Chem. Commun.*, 1983, 652.



(31)



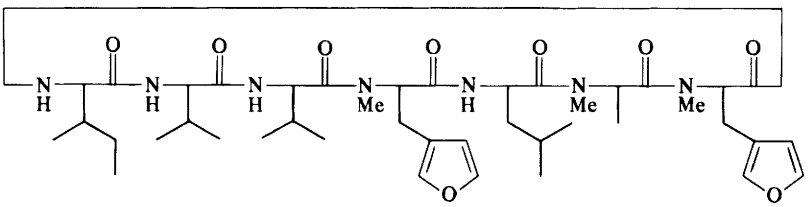
(32) a; $R^1 = R^2 = R^3 = H$
b; $R^1 = Me$, $R^2 = R^3 = H$
c; $R^1 = Me$, $R^2 = H$, $R^3 = OH$
d; $R^1 = Me$, $R^2 = OH$, $R^3 = H$

have been isolated from the roots of *Rubia cordifolia* and *Rubia akane*, two aromatic side chains being linked through an ether bridge. One component (32a) was examined by *X*-ray as its *p*-bromobenzoate, and it was found to be identical to deoxybouvardin, described in 1977.[49]

Cyclo[Cys(Acm)-D-Leu-His-Cys(Acm)-D-Leu-His] has been prepared by an azide cyclization, the D-leucines serving to place the imidazolyl and acetamido groups on the same side of the cyclic molecular plane. ^{1}H n.m.r. suggests that two β-turns are formed in the Leu-His sequences and that there are transannular H bonds between the cysteine residue amide bonds. Cu^{II} appears to interact with the His imidazoles but not the Acm (*S*-acetamidomethyl) groups. Zn^{II} co-ordinates with the peptide in a mode different from that of Ca^{II}.[50] A series of cyclic hexapeptides cyclo(Xxx-Pro-D-Gln)$_2$ (Xxx = Gly, Ala, Leu, Phe, or Val) have been elaborated. The best general route to these involved a diphenylphosphoryl azide cyclization, giving 36–46% of cyclic hexapeptide and 4–5% of cyclic [12]-peptide. A ^{1}H n.m.r. study of the NH region of the Leu and Val members of the series showed an unsymmetrical backbone, presumed to have one *cis*-Xxx—Pro bond, to be important.[51]

A two-dimensional n.m.r. study of the two cyclohexapeptides cyclo[Phe-D-Trp-Lys(*Z*)-Thr-Xxx-Pro] (Xxx = Gly or Phe) in DMSO shows dramatic conformational differences. When Xxx is Phe, there is a βII and a βVI turn in the molecule, with a *cis*-Phe—Pro peptide bond. In the Gly peptide, two species were observed in slow equilibrium (70:30), the major one involving a *trans*-Gly—Pro bond. This paper describes the technique of relayed heteronuclear correlation spectroscopy and a new improved pulse sequence that increases sensitivity.[52] [5-β-Cyanoalanine]- and [9-α-aminoacetonitrile]-oxytocin have been prepared; the former has <3% of the activity of oxytocin, but the latter has high activity, suggesting that the 5- and 9-carboxamide groups interact differently with reception sites.[53]

Rhozonin A, a novel cyclic heptapeptide (33) metabolite from *Rhizopus microsporus*, is the first mycotoxin to be reported from the order Mucoreles of



(33)

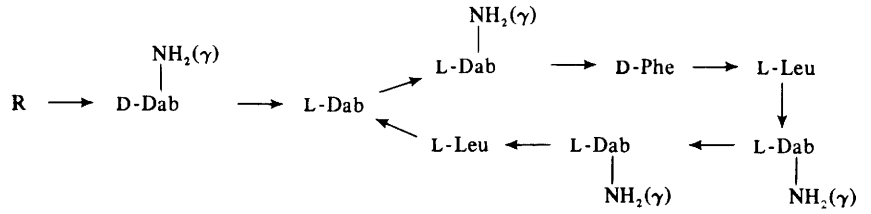
[49] H. Itokawa, K. Takeya, K. Mihara, N. Mori, T. Hananaka, T. Sonabe, and Y. Iitake, *Chem. Pharm. Bull.*, 1983, **31**, 1424.

[50] M. Kodaka, T. Shimizu, and M. Hatano, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 523, 1181.

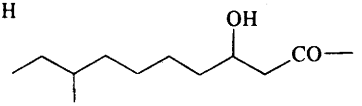
[51] K. D. Kopple and K. N. Parameswaran, *Int. J. Pept. Protein Res.*, 1983, **21**, 269.

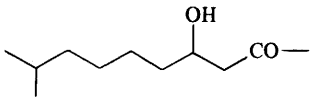
[52] H. Kessler, M. Bend, M. Kogler, J. Zarbock, D. W. Sørensen, G. Bodenhausen, and R. R. Ernst, *J. Am. Chem. Soc.*, 1983, **105**, 6944.

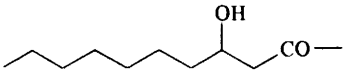
[53] U. Roy, D. Gazis, G. Dalpan, I. L. Schwartz, and J. Roy, *Int. J. Pept. Protein Res.*, 1983, **22**, 525.



(34) C_0, R = H

C_2, R = 

C_3, R = 

C_4, R = 

the lower fungi. Isolated from a strain growing on Mozambican groundnuts, it contains two residues of *N*-methyl-3-(3-furyl)alanine (MeFur). *X*-Ray analysis shows the two Val residues, the two MeFur residues, and Leu and a-Ile to have opposite configurations. Rhozonin B was also sequenced and differs only in the substitution of a-Ile by Val.[54] The structures of four components of an antibiotic complex Bu-2470 produced by *Bacillus circulans*, the octapeptins (34), have been determined. They differ only in the β-hydroxy C_{11} or C_{10} fatty acid acylating the pendant amino acid, the cyclic hexapeptide ring being invariant and identical to that earlier established for octapeptin C_1.[55] Three cyclic octapeptides of the type cyclo(Xxx-Pro)$_4$ [Xxx = Phe, Leu, or Lys(*Z*)] have been prepared by 1-succinimidyl ester cyclization in pyridine. The number of available conformations in solution due to *cis–trans* isomerization of Pro peptide bonds was found to depend on the nature of the solvent and the Xxx residue. They decreased in $CDCl_3$ in the following order: Lys(*Z*) > Leu > Phe. In alcohol the Phe cyclic peptide did not form complexes but the other two did selectively with Ba^{II} and Ca^{II}. In CD_3CN the Phe compound will complex with Ba^{II}.[56]

A number of gramicidin S (GS) analogues have been reported. [*O*-Bzl-D-Ser$^{4,4'}$]-GS has anti-microbial activity as high as that of GS itself, but after debenzylation the activity was low. Successive treatment of the resulting [D-Ser$^{4,4'}$]-GS with 2-t-butyloxycarbonylimino-2-phenylacetonitrile and DCCI–CuCl gave [L-Orn(δ-Boc)$^{2,2'}$,Δ-Ala^4,D-Ser$^{4'}$]-GS. Dehydration of the second serine

[54] P. S. Steyn, A. A. Tuinman, F. R. van Heerden, P. H. van Rooyen, P. L. Wessels, and J. C. Rabie, *J. Chem. Soc., Chem. Commun.*, 1983, 47.

[55] K. Sugawara, T. Yonemoto, M. Konishi, K. Matsumoto, T. Miyaki, and H. Kawaguchi, *J. Antibiot.*. 1983, **36**, 634.

[56] S. Kimura and Y. Imanishi, *Biopolymers*, 1983, **22**, 2191, 2383.

residue could not be effected without the peptide ring opening. In the presence of Palladium Black, the Δ-Ala residue was asymmetrically hydrogenated to D-Ala.[57] [$Pro^{4,4'}$,$Asn^{5,5'}$]-GS and [$Pro^{4,4'}$,D-$Ala^{5,5'}$]-GS have been prepared to investigate the possibility of replacing the type II′ β-turn by a different type of β-turn, but neither analogue showed antibiotic activity.[58] Five GS analogues have been synthesized by the solid-phase method to evaluate the roles of the Val and Orn residues. The compounds [D-$Tyr^{4,4'}$]-GS, [Gly^{1}]-GS, and [Hnv^{2}]-GS (Hnv = δ-hydroxy-L-norvaline) showed high anti-bacterial activities, but [$Gly^{1,1'}$]-GS and [$Hnv^{2,2'}$]-GS were inactive.[59]

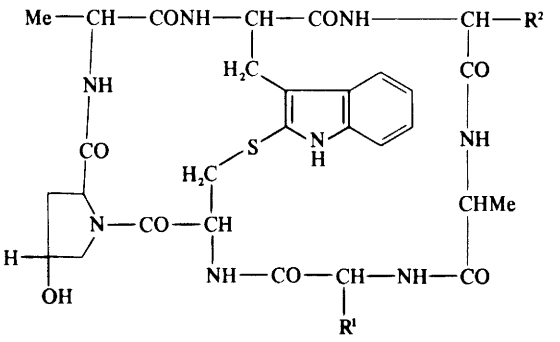
[$Orn(\delta\text{-}Z)^{2,2'}$]-GS containing two residues of (2*R*,3*R*)-phenylalanine-2,3-d_2 has been prepared, and its ^{1}H n.m.r. spectra have been examined. The pro-*R* and pro-*S* protons resonate at 3.0 and 2.9 p.p.m., respectively, whereas these values are reversed in GS itself. This chemical-shift reversal is attributed to steric crowding round the D-Phe—Pro peptide bonds by the Z-groups, leading to rotation of the aromatic side chains of the D-Phe residues.[60] GS has been found to bind nucleotides in water to yield a complex that partitions into organic solvents. This appears to be the first instance of a peptide possessing such phase-transfer activity towards nucleotides, and the property may be related to the antimicrobial activity of GS.[61]

[6-(L-2,6-Diamino-4-hexynoic acid)]-antamanide has been synthesized as a precursor of ^{3}H-labelled [Lys^{6}]-antamanide required for photoaffinity labelling.[62]

The natural cyclo[12]-peptide gratisin, cyclo(Val-Orn-Leu-Phe-Pro-Tyr)$_2$, was described in 1973, but the amino acid configurations have not yet been established. Synthetic all-L gratisin showed little of the antibiotic activity of the natural material, but [D-$Phe^{4,4'}$]-gratisin is quite active. [D-$Phe^{4,4'}$,D-$Tyr^{6,6'}$]-gratisin has now been prepared and been shown to be highly active. It is concluded that this compound is likely to be identical to the natural product.[63] Peptide-proton and side-chain carboxamide proton-exchange rates of cyclo(Met-Val-Gly-Pro-Asn-Gly)$_2$ have been determined over the pH range 1.2–9 by n.m.r. methods. The side-chain exchange rates in acid are similar to the corresponding rates in model substances, but the rates are 10–20 times slower for the backbone protons. It is suggested that backbone conformational stability provides a barrier to the rehybridization at nitrogen necessary for exchange *via N*-protonation. The base-catalysed rates are consistent with the proposed conformation for this cyclic peptide.[64]

Cyclic Peptides from *Amanita* Species. — The analogues [D-Abu^{2},Lys^{7}]-phalloin (35a) and [D-Ala^{2},Leu^{7}]-phalloin (35b) have been prepared, using the earlier

[57] S. Ando, H. Aoyagi, M. Waki, T. Kato, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1983, **21**, 313.
[58] K. Sato and U. Nagai, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 3329.
[59] H. Yonezawa, K. Okamoto, M. Kaneda, N. Tominaga, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1983, **22**, 573.
[60] K. Tanimura, T. Kato, M. Waki, and N. Izumiya, *Tetrahedron Lett.*, 1983, **35**, 3737.
[61] E. M. Krauss and S. I. Chan, *Biochemistry*, 1983, **22**, 4280.
[62] M. Nassal, P. Buc, and Th. Wieland, *Liebigs Ann. Chem.*, 1983, 1524.
[63] M. Tamaki, M. Takimoto, S. Sofuku, and I. Muramatsu, *J. Antibiot.*, 1983, **36**, 751.
[64] V. P. Narutis and K. D. Kopple, *Biochemistry*, 1983, **22**, 6233.

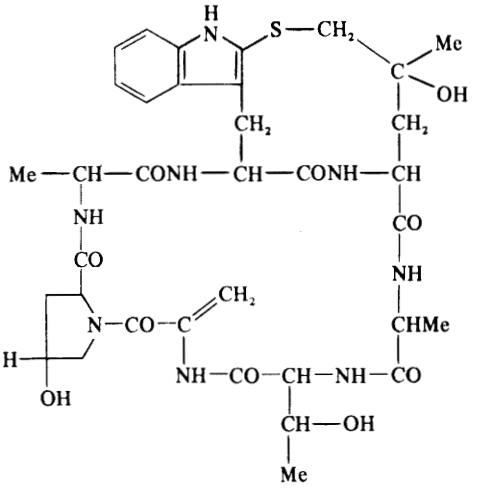


(35) a; R^1 = Et, R^2 = $-(CH_2)_4NH_2$
b; R^1 = Me, R^2 = $-CH_2CHMe_2$
c; R^1 = $-CH(OH)Me$, R^2 = $-CH_2C(OH)(Me)-CH_2-O$

reported methods of cyclization. The former binds strongly to F-actin, but the latter has little affinity. Treatment of (35a) with tetramethylrhodaminylisothiocyanate forms a product that is a good red-fluorescing stain for F-actin in various cell preparations.[65] *X*-Ray analysis of 6′-*O*-Me-α-amanitin-(*S*)-sulphoxide and the corresponding sulphone shows practically identical conformations. N.m.r. spectroscopy in DMSO confirms this result, the structures closely resembling that of the (*R*)-sulphoxide. This result is surprising in view of the lack of high toxicity in the (*S*)-sulphoxide.[66]

Tosylation of phalloidin gives the $O^{\delta 7}$-tosyl derivative (35c) in which the primary alcoholic group of the γ,δ-dihydroxyleucine side chain is substituted. The tosyl group can be replaced by ammonia, aniline, or 4′-aminofluorescein, giving new phalloidin analogues.[67] Treatment of $O^{\delta 7}$-tosyl phalloidin with strong bases leads to loss of the tosyl group and rearrangement of the thioether linkage to give (36) (presumably by alkylation of the sulphur and subsequent β-elimination of the sulphonium ion); the c.d. spectrum indicates an inversion of the helicity of the thioether chromophore in this process.[68] Periodate cleavage of the γ,δ-dihydroxyleucine residue of α-amanitin without prior methylation of the 6′-OH of the tryptophan affords α-amanitin aldehyde in 45% yield. The product is an active inhibitor of RNA polymerase II, as is its 2,4-DNP derivative. Replacement of the hydroxymethyl group by a more bulky residue therefore does not destabilize the amatoxin-RNA polymerase complex.[69] The 7′-position of the tryptophan residue of α-amanitin can be substituted by a phenylazo group

[65] Th. Wieland, T. Miura, and A. Seeliger, *Int. J. Pept. Protein Res.*, 1983, **21**, 3.
[66] Th. Wieland, C. Gotzendorfer, J. Dabrowski, W. N. Lipscomb, and G. Shoham, *Biochemistry*, 1983, **22**, 1264.
[67] Th. Wieland, M. Hollosi, and M. Nassal, *Liebigs Ann. Chem.*, 1983, 1533.
[68] Th. Wieland, M. Nassal, T. Miura, and C. Gotzendorfer, *J. Am. Chem. Soc.*, 1983, **105**, 6193.
[69] P. W. Morris and R. McSwine, *Int. J. Pept. Protein Res.*, 1983, **22**, 597.

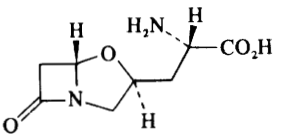


(36)

using benzenediazonium acetate in methanolic NaOH[70] and by the nitrogen of morphine using a complex of morpholine and iodine.[71] In both cases RNA polymerase binary complex formation is again unaffected by such substitution.[70,71]

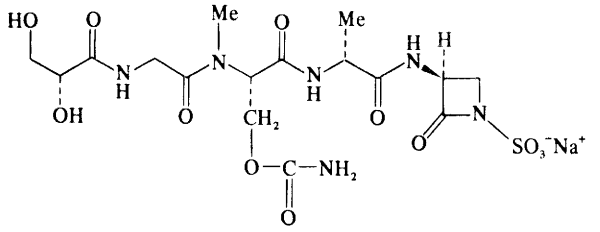
The fifty years' work on the toxic peptides from *Amanita* mushrooms has been authoritatively reviewed.[72]

Highly Modified Cyclic Peptides. — A new β-lactam antibiotic (37) from *Streptomyces clavuligerus*, a source earlier of several other antibiotics, acts as an inhibitor in methionine synthesis. The authors of this paper postulate that clavams require the *R*-configuration at the ring junction for β-lactamase inhibitory activity, while *S*-stereochemistry is essential for anti-fungal activity.[73] A new monobactam (38) from *Flexibacter* sp. shows weak anti-bacterial activity. G.l.c. of the *N*-pentafluoropropionyl isopropyl esters of the constituent fragments on

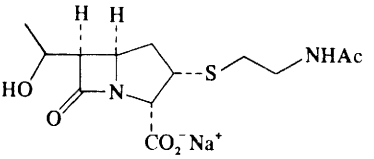


(37)

[70] E. Falck-Pederson, P. W. Morris, and D. L. Venton, *Int. J. Pept. Protein Res.*, 1983, **21**, 431.
[71] P. W. Morris and D. L. Venton, *Int. J. Pept. Protein Res.*, 1983, **21**, 419.
[72] Th. Wieland, *Int. J. Pept. Protein Res.*, 1983, **22**, 257.
[73] R. H. Evans, H. Ax, A. Jacoby, T. H. Williams, E. Jenkins, and J. P. Scannell, *J. Antibiot.*, 1983, **36**, 213.

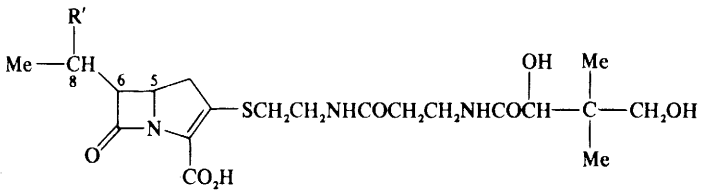


(38)



(39)

a chiral column showed the D-configurations for glyceric acid and alanine but L-stereochemistry for *N*-methylserine and 2,3-diaminopropionic acid.[74] A compound (39) possibly involved in the biosynthesis of carbapenem antibiotics has been isolated from culture filtrates of three *Streptomyces* strains isolated from soil samples and also from several other known antibiotic producers. It has no anti-bacterial activity or β-lactamase inhibitory activity.[75] Various *Streptomyces* sources have also yielded carbapenems with pantatheinyl side chains at C-3 (40),[76] two new carpetimycins (41),[77] and 8-*epi*-thienamycin (42a) and nor-



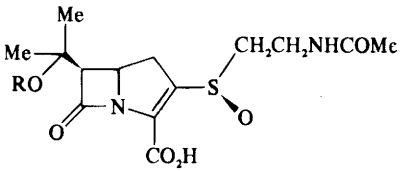
	R′	C-5	C-6	C-8	*Pantoyl*
A	H	R	R	–	R
B	OH	R	R	N.D.	R
C	OH	R	S	S	R
D	OSO_3H	R	R	N.D.	R

(40)

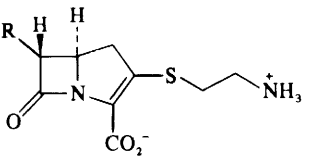
[74] P. D. Singh, J. H. Johnson, P. C. Ward, J. Scott Wells, W. H. Trejo, and R. B. Sykes, *J. Antibiot.*, 1983, **36**, 1245.

[75] T. Haneishi, M. Nakajima, N. Serizawa, M. Inukai, T. Takiguchi, M. Arai, S. Sato, H. Kuwano, and C. Tamura, *J. Antibiot.*, 1983, **36**, 1581.

[76] T. Yoshioka, I. Kojima, K. Isshiki, A. Watanabe, Y. Shimauchi, M. Okabe, Y. Fukagawa, and T. Ishikura, *J. Antibiot.*, 1983, **36**, 1473.

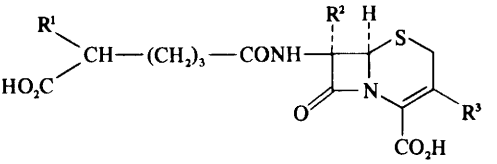


(41) C; R = H
D; R = —SO_3H



(42) a; R = 

b; R = —CH_2OH



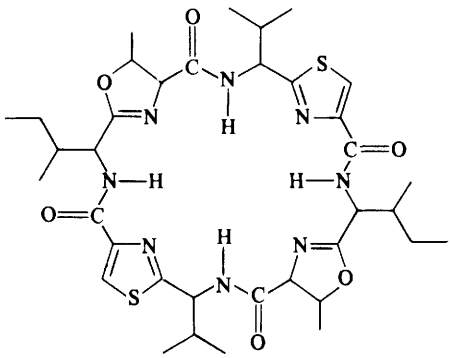
(43) a; $R^1 = NH_2$, $R^2 = OMe$, $R^3 = —COSSO_3H$ (SF 1623)
b; $R^1 = NH_2$, $R^2 = OMe$, $R^3 = —CO_2H$ (SF 1623B)
c; $R^1 = OH$, $R^2 = H$, $R^3 = —CH_2OH$

thienamycin (42b).[78] The last named is the first example of a carbapenem antibiotic with a one-carbon side chain at C-6.

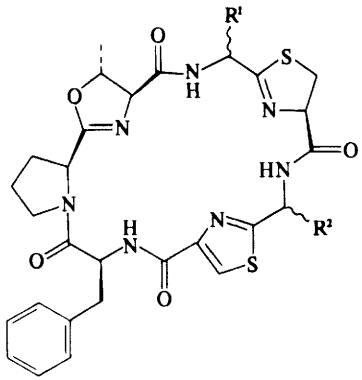
Two novel cephamycins, (43a) and (43b), have been isolated from *Streptomyces chartreusis*. The former is highly active against Gram-negative bacteria, and its production is stimulated by the presence of sodium thiosulphate in the growing medium.[79] The acidic antibiotic (43c) has also been obtained from a *Streptomyces* strain, PA-41937.[80]

A new cytotoxic cyclic peptide ascidiacyclamide (44) has been extracted from an unidentified species of ascidian collected from Rodda Reef, Queensland. Owing to the symmetric structure, only 18 ^{13}C and 26 ^{1}H n.m.r. signals were observed. It contains the oxazoline and thiazole rings previously observed in ulithiacyclamide (see Volume 13 of this title, p. 306).[81] The tunicate *Lissoclinum patella*, from which ulithiacyclamide, ulicyclamide, and the patellamides were isolated earlier, has now yielded three further cyclic peptides (45a, b, and c). The two epimers represent the first thiazoline-containing peptides in the lissoclinum series. The similarity of their spectral properties to those of ulicyclamide has led

[77] M. Nakayama, S. Kimura, T. Mizoguchi, S. Tanabe, A. Iwasaki, A. Murakami, M. Okuchi, H. Itoh, and T. Mori, *J. Antibiot.*, 1983, **36**, 943.
[78] K. E. Wilson, A. J. Kempe, J. M. Liesch, and B. H. Arison, *J. Antibiot.*, 1983, **36**, 1109.
[79] S. Inouye, M. Kojima, T. Shomura, K. Iwomatsu, T. Nira, Y. Kando, T. Niida, Y. Ogawa, and K. Kusama, *J. Antibiot.*, 1983, **36**, 115.
[80] J. Shoji, R. Sakazaki, K. Matsumoto, T. Tanimoto, Y. Terui, S. Kozuku, and E. Kondo, *J. Antibiot.*, 1983, **36**, 167.
[81] Y. Hamamoto, M. Eudo, M. Nakagawa, T. Nakanishi, and K. Mizukawa, *J. Chem. Soc., Chem. Commun.*, 1983, 323.



(44)

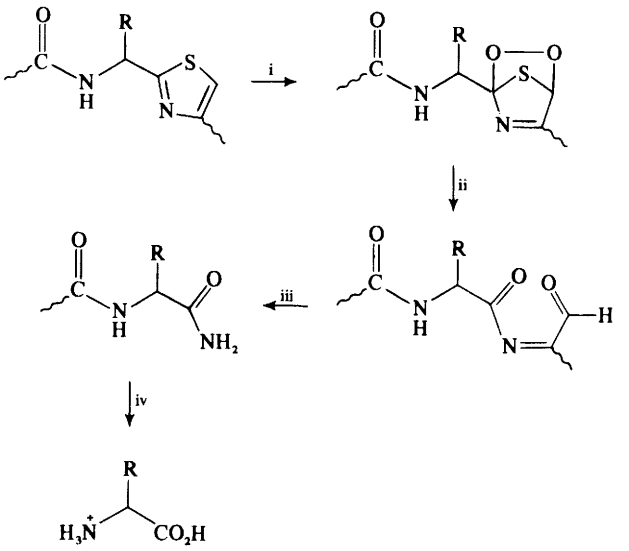


(45) a; R^1 = —CMe_2; R^2 = ···CHMeEt
b; R^1 = ···CHMeEt; R^2 = ···Me
c; R^1 = ···CHMeEt; R^2 = —Me
d; R^1 = —CHMeEt; R^2 = ···Me

to the structure of the latter being revised to (45d).[82] A general and mild method for determining the chirality of thiazole amino acids in peptides has been developed, based on the [4 + 2] cycloaddition of thiazoles with singlet oxygen (Scheme 14). When applied to the lissoclinum peptides it showed them to possess predominantly the *R*-configuration.[83]

A ^{1}H n.m.r. analysis of thiostrepton agrees with earlier evidence that the side chain terminates in two dehydroalanine residues, as indicated in (46a), and that the overall conformation in $CDCl_3$ is similar to that found in the crystal form.[84] A similar study of the thiopeptins, a closely related group of compounds produced by *Streptomyces tateyamensis*, has elucidated the structures of four

[82] J. M. Wasylyk, J. E. Biskupiak, C. E. Costello, and C. M. Ireland, *J. Org. Chem.*, 1983, **48**, 4445.
[83] J. E. Biskupiak and C. M. Ireland, *J. Org. Chem.*, 1983, **48**, 2302.
[84] O. D. Hensens, G. Albers-Schönberg, and B. F. Anderson, *J. Antibiot.*, 1983, **36**, 799.



Reagents: i, $^1O_2$; ii, MeOH; iii, H_2O; iv, 6M HCl, 120 °C

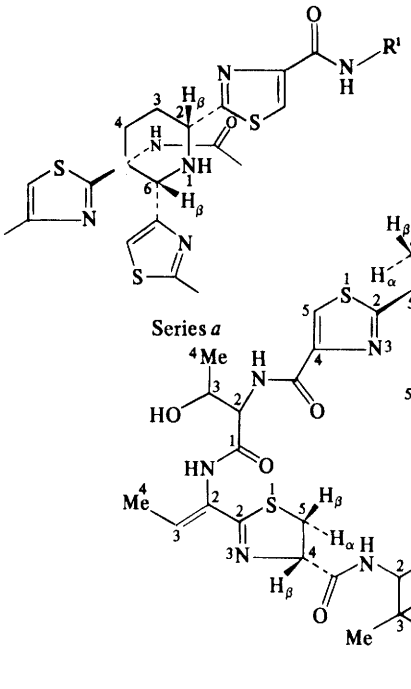
Scheme 14

components (46b–e). As separated by t.l.c., mixtures of compounds containing piperidine (Series *b*) and tetrahydropyridine (Series *a*) rings at the side-chain–macrocycle linkage were obtained. These may be resolved by h.p.l.c. These molecules were not amenable to m.s. and *X*-ray analysis. The major component, thiopeptin B, is valuable as a feed additive because of its growth-promoting action in swine and chickens.[85] A detailed ^{13}C n.m.r. study of thiostrepton and the thiopeptins is consistent with their proposed structures.[86]

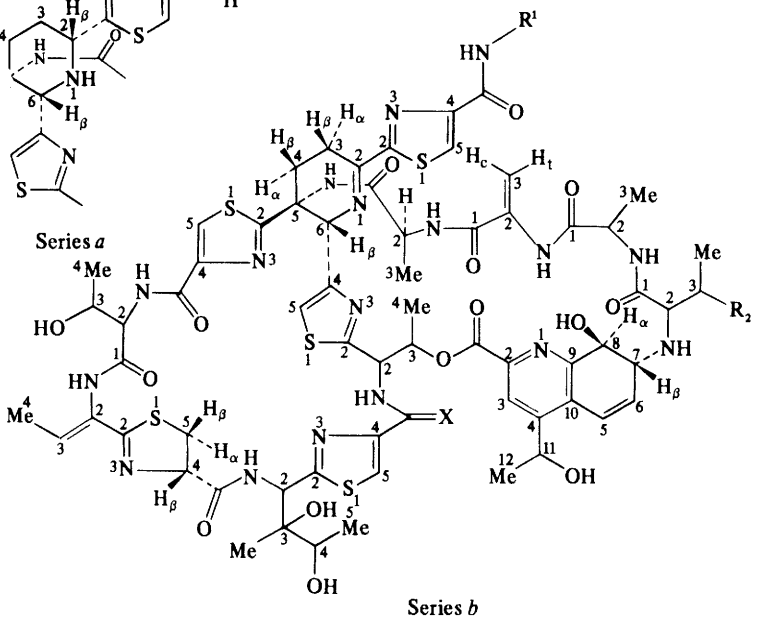
3 Cyclic Depsipeptides

Treatment of Boc-Ser-Phe-Pro-ONp with NaH in DMF gives a mixture of the ten-membered cyclodepsipeptide (47) together with two other products (Scheme 15). With mild alkaline buffer only azacyclol (48) is formed. In an attempt to form a six-membered ring oxacyclol, *RS*-3-hydroxybutyryl-Phe-Pro-ONp, was treated with both mild and strong base. Under both conditions a mixture of cyclodepsipeptide (49) and the two epimeric β-hydroxyacyl-2,5-dioxopiperazines was formed, no six-membered oxacyclol being produced. On hydrazinolysis (47) gave predominantly Boc-Ser-Phe-Pro-$NHNH_2$ whereas (49) gave cyclo(Phe-Pro) as the main product. This indicates an easy isomerization of (49) into the corresponding *N*-acyldioxopiperazine through an intermediate six-membered oxacyclol, but (47) undergoes direct attack by the hydrazine on the lactone carbonyl.[87]

[85] O. D. Hensens and G. Albers-Schönberg, *J. Antibiot.*, 1983, **36**, 814.
[86] O. D. Hensens and G. Albers-Schönberg, *J. Antibiot.*, 1983, **36**, 832.

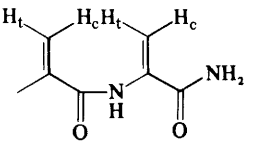


Series *a*



Series *b*

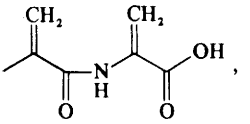
(46) a; Thiostrepton A_1: X = O, R^1 =



R^2 = Et

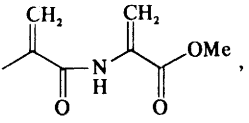
Deala (2) Deala (3)

b; Thiopeptin B_a/B_b: X = S, R^1 =



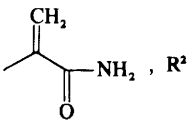
, R^2 = Me

c; Thiopeptin A_{1a}/A_{1b}: X = S, R^1 =



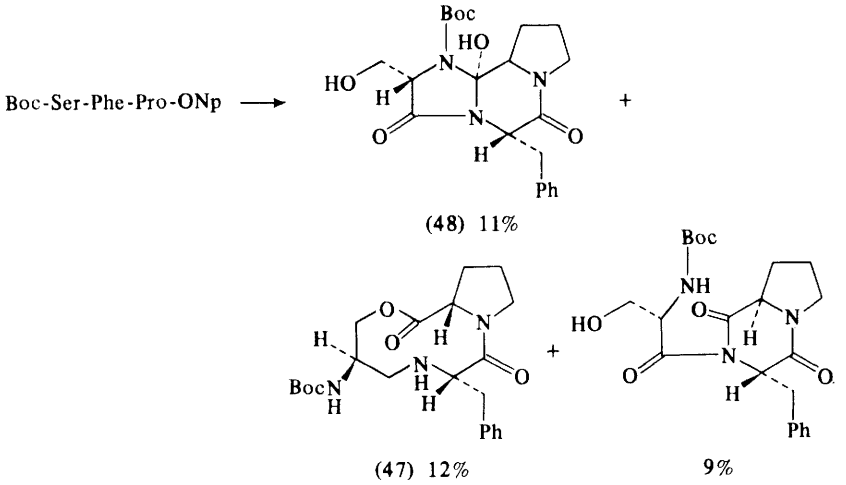
, R^2 = Me

d; Thiopeptin A_{4a}/A_{4b}: X = S, R^1 =

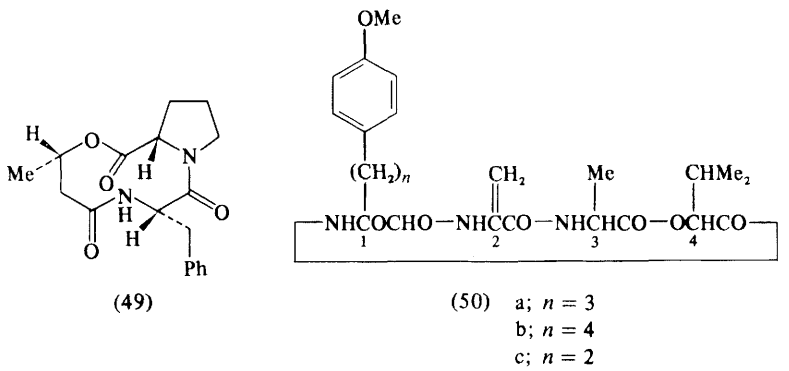


, R^2 = Me

e; Thiopeptin A_{3a}/A_{3b}: X = S, R^1 = H, R^2 = Me



Scheme 15



(49)

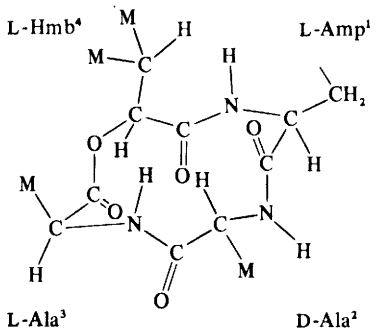
(50) a; $n = 3$
b; $n = 4$
c; $n = 2$

Two analogues of the cyclotetradepsipeptide AM toxin I (50a) have been prepared containing higher (50b) and lower (50c) homologues of the aromatic residue. Their weak necrotic activities on apple leaf indicate the high importance of the length of the side chain in this particular residue.[88] An AM toxin II analogue with α-hydroxyalanine (HyAla) in place of dehydroalanine has been prepared by intramolecular addition in HF of pyruvoyl-Ala-Hmb-App-NH_2 (Hmb = 2-hydroxy-3-methylbutanoic acid, App = 2-amino-5-phenylpentanoic acid). A singlet peak for the α-OH in the ^1H n.m.r. spectrum suggests that the HyAla is of a single optical configuration. The lack of toxicity of this analogue towards apple leaves indicates that there is no biological transformation of

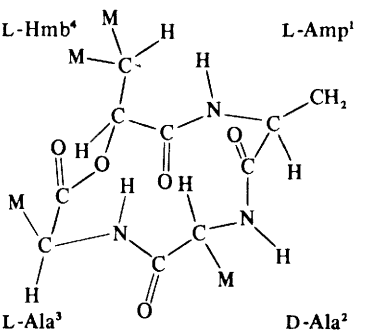
[87] G. Zanotti, F. Pinnen, G. Lucente, S. Cerrini, E. Gaivuzzo, and F. Mozza, *Int. J. Pept. Protein Res.*, **1983**, **22**, 410.

[88] H. Mihara, H. Aoyagi, T. Kato, T. Ueno, and N. Izumiya, *Chem. Lett.*, **1983**, 811.

HyAla to ΔAla.[89] [D-Ala^2]-AM toxin I and [L-Ala^2]-AM toxin I have been prepared; the former had necrotic activity, but the latter was non-toxic. A 270 MHz ^{1}H n.m.r. study of these analogues and AM toxins I and II showed for the natural materials the presence of two conformers (51) and (52) but a single conformer for each of the synthetic materials. For [D-Ala^2]-AM toxin I this corresponds to the major conformer (51) and thus appears to be related to toxicity.[90]



(51)

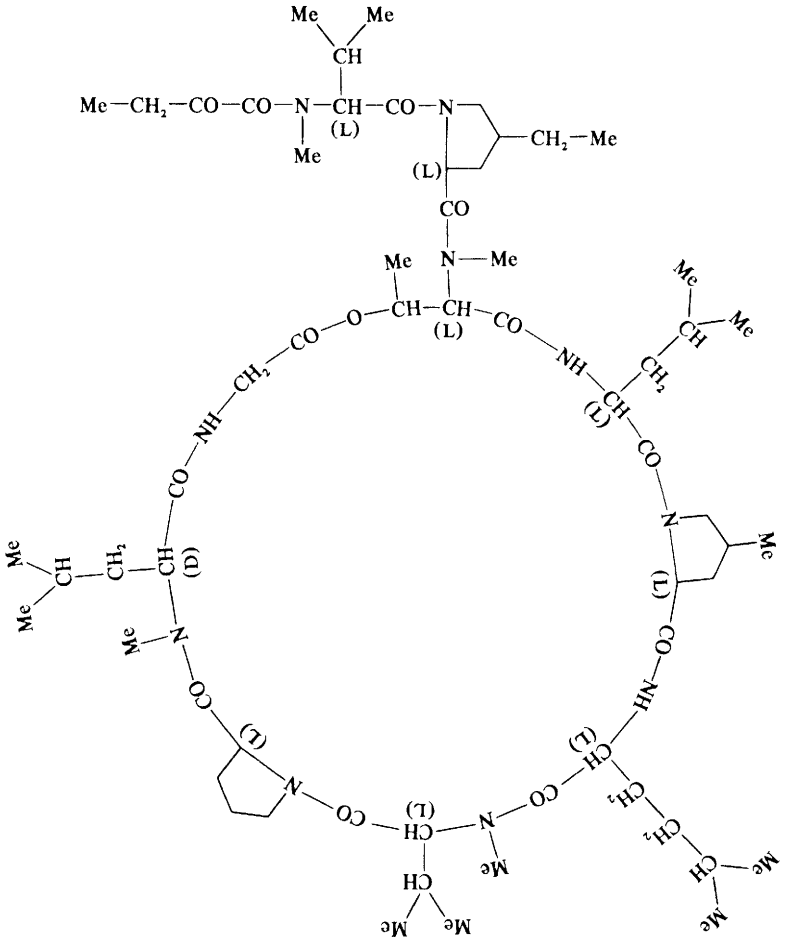


(52)

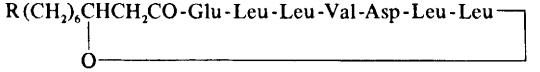
The enzyme enniatin synthetase from *Fusarium oxysporum* has been found to catalyse formation of the dipeptide D-2-hydroxyisovaleryl-*N*-methylvaline, which then further condenses to give the cyclohexadepsipeptide enniatin.[91] A new cyclo-octadepsipeptide antibiotic mycoplanecin A (53) has been isolated from *Actinoplanes awajinensis*. It shows specific activity against mycobacteria and is closely related to griselimycin. Ethyl-L-proline, one of its constituents, has not been recorded before as a component of a natural product.[92] The structures of a series of inhibitors of cyclic adenosine 3′,5′-monophosphate phosphodiesterase have been elucidated, largely by mass spectrometry. These metabolites of *Bacillus subtilis* C-756 (54) differ only in the β-hydroxy fatty acid, and the amino acid sequence is identical to that of esperin, although in the latter the lactone ring involves the β-carboxyl of the Asp instead of the Leu.[93]

F.a.b. m.s. has proved more successful than the field-desorption method in determining the molecular weights of a number of new antibiotics of the triostin group that have both the natural quinoline-2-carboxyl chromophores replaced by substituted analogues. These were prepared by supplementing the culture media with the appropriate quinoline carboxylic acids.[94] The n.m.r. pattern of

[89] K. Noda, J. Nakashima, S. Lee, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1245.
[90] T. Higashijima, Y. Shimohigashi, T. Kato, N. Izumiya, T. Ueno, and T. Miyazawa, *Biopolymers*, 1983, **22**, 1167.
[91] R. Zocher, U. Keller, and H. Kleinkauf, *Biochem. Biophys. Res. Commun.*, 1983, **110**, 292.
[92] M. Nakajima, A. Torikata, H. Tamaoki, T. Haneishi, and M. Arai, *J. Antibiot.*, 1983, **36**, 967.
[93] K. Hosono and H. Suzuki, *J. Antibiot.*, 1983, **36**, 674.
[94] S. Santikarn, S. J. Hammond, D. H. Williams, A. Cornish, and M. J. Waring, *J. Antibiot.*, 1983, **36**, 362.



(53)

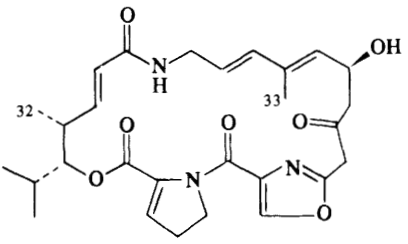


(54) R = Me_2CHCH_2—, EtMeCH—, $Me(CH_2)_4$—, Me_2CH—, or $EtMeCH(CH_2)_2$—

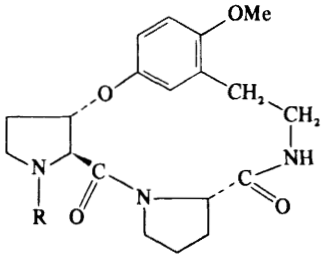
triostin A in weakly polar solvents has been interpreted in terms of two symmetrical conformers. Their slow interconversion is thought to arise from *cis–trans* isomerization of the *N*-methyl peptide bonds. Only one of the two forms interacts with adenosine and guanosine derivatives.[95]

[95] N. Noguchi, Y. Kyogoku, M. Shin, and K. Inouye, *Int. J. Pept. Protein Res.*, 1983, **21**, 541.

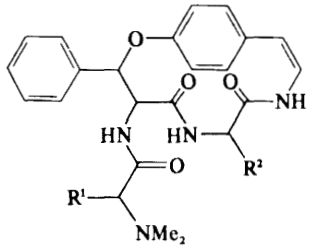
A new n.m.r. method for the simultaneous detection of unique hydrogen-bonded atom pairs that does not introduce perturbations into the molecular system has been demonstrated on valinomycin. The calculated length of the D-ValNH and L-Lac C=O hydrogen bond (2.22 Å) is smaller than that found in the crystal (2.95 Å).[96] The biosynthesis of the highly modified depsipeptide antibiotic virginiamycin (55) has been investigated by feeding with ^{13}C-labelled precursors. The basic skeleton is derived from seven acetate units, valine, proline, glycine, and serine, the latter giving rise to the oxazole ring. The C-32 methyl group is derived from methionine, but that at C-33 is derived by a new pathway involving decarboxylation of an acetate unit.[97]



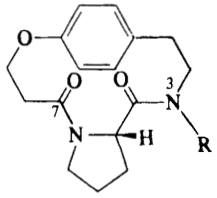
(55)



(56) a; R = Me_2Ile-Ile-
b; R = MeIle-Ile-



(57) C; $R^1 = R^2$ = isobutyl
D; R^1 = isobutyl, R^2 = benzyl



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

4 Peptide Alkaloids

Full details of the total synthesis of zizyphine A have now appeared.[98] The 10,11-dihydrozizyphines A (56a) and B (56b) have been synthesized, again using catalytic hydrogenation to effect ring closure of linear ω-Z-amino acid penta-fluorophenyl esters.[99] Two new peptide alkaloids discarines C and D (57) have

[96] M. A. Khaled and C. L. Watkins, *J. Am. Chem. Soc.*, 1983, **105**, 3363.
[97] D. G. I. Kingston, M. X. Kolpak, J. W. Le Fevre, and I. Borup-Grochtmann, *J. Am. Chem. Soc.*, 1983, **105**, 5106.
[98] U. Schmidt, A. Lieberknecht, H. Bokens, and H. Griesser, *J. Org. Chem.*, 1983, **48**, 2860.
[99] U. Schmidt, H. Bokens, A. Lieberknecht, and H. Griesser, *Liebigs Ann. Chem.*, 1983, 1459.

been isolated from *Discaria febrifuga*.[100] The *X*-ray crystal structure of a synthetic dihydro-*p*-phencyclopeptine (58a) shows both amide bonds to be *trans*, with the carbonyls pointing to opposite sides of the macrocycle. The solution conformation appears to be nearly identical, but that of the *N*-desmethyl compound (58b) is subtly different near the amide bond involving the proline carbonyl. This is thought to be due to the formation of a hydrogen bond between the N-3—H and the C-7 carbonyl group.[101]

5 Linear Peptides

Peptides Containing α-Aminoisobutyric Acid. — Leucinostatin B from *Paecilamyces lilacinus* has now been sequenced. It differs from leucinostatin A, whose structure was given in Volume 15 (p. 393), in only two respects. It possesses an extra Aib residue before the β-alanine residue, and the acyl group at the N-terminus is of opposite configuration.[102] [Aib[1]]-angiotensin II has been synthesized by the solid-phase method. This analogue is significantly more active than the parent molecule.[103] [Aib[2]]- and [Aib[2,3]]-enkephalins have proved to have high *in vivo* biological activity, but [Aib[3]]-enkephalin is less active. The disubstituted peptide adopts a 3_{10}-helical conformation, but the monosubstituted ones contain β-turns in solution.[104]

X-Ray crystal analyses of peptides rich in Aib residues have been so numerous this year that they can only be listed (Table 2) without individual comment.[105–116] In general they confirm the strong tendency of Aib residues to promote 3_{10}-helix formation. The conformational preferences of linear peptides

[100] M. Digel, A. March, H. Layer, J. Biermarn, and W. Voelter, *Hoppe-Seyler's Z. Physiol. Chem.*, 1983, **364**, 1641.
[101] J. C. Lagarias, W. H. Yakoyama, J. Bardner, W. C. Shik, M. P. Klein, and H. Rapaport, *J. Am. Chem. Soc.*, 1983, **105**, 1031.
[102] Y. Mori, M. Suzuki, K. Fukushima, and T. Arai, *J. Antibiot.*, 1983, **36**, 1086.
[103] P. Cardopatis and D. Theodoropoulos, *Experientia*, 1983, **39**, 106.
[104] T. S. Sudha and P. Balaram, *Int. J. Pept. Protein Res.*, 1983, **21**, 381.
[105] R. Bosch, K.-P. Voges, G. Jung, and W. Winter, *Acta Crystallogr., Sect. C*, 1983, **39**, 481.
[106] P. Van Roey, G. D. Smith, T. M. Balasubramanian, and G. R. Marshall, *Acta Crystallogr., Sect. C*, 1983, **39**, 894.
[107] C. Toniolo, G. M. Bonora, M. Crisma, E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, and C. Pedone, *Int. J. Pept. Protein Res.*, 1983, **22**, 603.
[108] E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo, G. M. Bonora, and M. Crisma, *Int. J. Pept. Protein Res.*, 1983, **22**, 385.
[109] P. Van Roey, G. D. Smith, T. M. Balasubramanian, E. W. Czerwinski, G. R. Marshall, and F. S. Mathews, *Int. J. Pept. Protein Res.*, 1983, **22**, 404.
[110] H. Balaram, B. V. V. Prasad, and P. Balaram, *J. Am. Chem. Soc.*, 1983, **105**, 4065.
[111] B. V. V. Prasad, T. S. Sudha, and P. Balaram, *J. Chem. Soc., Perkin Trans. 1*, 1983, 417.
[112] A. Ravi, B. V. V. Prasad, and P. Balaram, *J. Am. Chem. Soc.*, 1983, **105**, 105.
[113] C. Toniolo, G. M. Bonora, E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, and C. Pedone, *Biopolymers*, 1983, **22**, 1335.
[114] A. K. Francis, M. Iqbal, P. Balaram, and M. Vijayan, *Biopolymers*, 1983, **22**, 1499.
[115] R. Bosch, G. Jung, and W. Winter, *Acta Crystallogr., Sect. C*, 1983, **39**, 776.
[116] G. Jung, R. Bosch, E. Katz, H. Schmitt, K.-P. Voges, and W. Winter, *Biopolymers*, 1983, **22**, 241.

Table 2 *Crystal structures of Aib peptides*

Peptide	*Ref.*
Boc-Ala-Aib-OH	105
Boc-Aib-Aib-OBzl	106
Z-$(Aib)_3$-OH oxazolone*	107
Z-Aib-Pro-Aib-OMe	108
Boc-Pro-Aib-Aib-OMe	109
Boc-Aib-Pro-Pro-NHMe*	110
Boc-Aib-Aib-Phe-Met-NH_2	111
Boc-Cys-Pro-Aib-Cys-NHMe*	112
Z-$(Aib)_3$-Val-Gly-OMe*	113
Boc-Ala-Aib-Ala-Aib-Aib-OMe	114
Boc-Aib-Ala-Aib-Ala-Aib-OMe	115, 116
Boc-$(Ala-Aib)_2$-Ala-Glu(OBzl)-$(Ala-Aib)_2$-Ala-OMe	116

* Indicates that spectroscopic studies in solution have also been carried out

Table 3 *Solution conformations of Aib peptides*

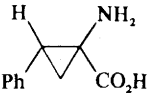
Peptide	*Techniques used*	*Ref.*
Piv-Pro-Aib-NHMe	N.O.e., c.d.	119
Z-$(Aib)_n$-OH oxazolones (n = 2–4)	I.r.	107
Boc-Cys-Pro-Aib-Cys-NHMe	N.O.e., c.d.	119
Boc-Val-$(Aib-Val)_2$-OMe	^{1}H n.m.r.	120
Boc-Ala-$(Aib-Ala)_3$-OMe	^{1}H n.m.r.	120
Boc-Val-$(Aib-Val)_3$-OMe	^{1}H n.m.r.	120
Z-$(Aib)_3$-Val-Gly-Leu-$(Aib)_2$-OMe and smaller fragments	I.r., c.d.	113
Boc-$(Aib-Ala)_5$-OMe	^{1}H n.m.r.	121, 122
Boc-$(Aib-Val)_5$-OMe	^{1}H n.m.r.	121, 122
Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-$(Aib)_2$-OMe	C.d.	122
Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-$(Aib)_2$-OMe	C.d.	122
Boc-Ala-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-$(Aib)_2$-OMe	C.d.	122
Boc-Ala-Aib-Ala-$(Aib)_2$-Gln-Aib-Leu-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-$(Aib)_2$-OMe	C.d.	122
Boc-Aib-Pro-Val-Aib-Val-$(Ala-Aib)_2$-Aib-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-$(Aib)_2$-OMe	C.d.	122
Alamethicin	500 MHz ^{1}H n.m.r.	123

containing α,α-disubstituted amino acids, derived from the crystal structures of 28 compounds, have been reviewed.[117] Electron-diffraction photographs of poly(Aib) treated with dichloroacetic acid to improve crystallinity indicate the formation of hexagonal cells in which RH and LH 3_{10}-helices form a honeycomb

[117] C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, D. Di Blasio, V. Pavone, and C. Pedone, *Biopolymers*, 1983, **22**, 205.

structure.[118] Conformational studies of Aib-containing peptides in solution have also been widely pursued this year; they are summarized in Table 3.[119–123]

Peptides Containing Other α,α-Disubstituted Amino Acids. – [D-Ala^2, Δ^EPhe^4, Leu^5]-enkephalin [Δ^EPhe is the *E*-isomer of 2-phenyl-1-aminocyclopropane carboxylic acid (59)] has been prepared, but it only has low activity on MVD and GPI assays.[124] The solid-phase synthesis of [cycloLeu^2]- and [penicillamine^1, cycloLeu^2]-oxytocin by the solid-phase method has been reported (cycloLeu is 1-aminocyclopentane carboxylic acid).[124a]

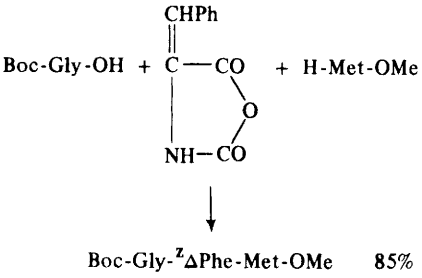


(59)

The incorporation of α,α-di-n-propylglycine (Dpg) residues into peptides has been examined in some detail. Steric hindrance is more severe than with Aib, but coupling to the amino group is easier than to the carboxyl. Of the conventional peptide-coupling methods, only activation of the Dpg carboxyl with 3-hydroxy-4-oxo-3,4-dihydrobenzotriazine was useful. The reagent of choice for introducing a Dpg residue proved to be 2-trifluoromethyl-4,4-dipropyloxazolin-5(4H)-one Using this reagent, Tfa-(Dpg)$_6$-OBu^t was prepared, removal of Tfa groups from intermediates requiring treatment with sodium borohydride. As the conditions for this deprotection tend to reduce even the t-butyl esters of protein amino acids, C-protection with the *N'*,*N'*-dibenzylhydrazide was developed. The free carboxyl group can be regenerated by treatment with bromine in acetonitrile.[125]

Peptides Containing Dehydroamino Acid Residues. – [Δ^2Phe^4]-enkephalin is nearly twice as active as enkephalin in an *in vitro* assay, but its analgesic potency is considerably lower *in vivo*.[126] The protected enkephalin derivatives Boc-ΔTyr-(Cl$_2$Bzl)-D-Ala(or Gly)-Gly-Phe-Leu-OBzl have been synthesized, but attempts to liberate [ΔTyr^1]-enkephalin have failed through the apparent instability of an

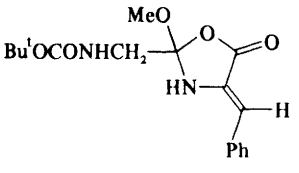
[118] B. R. Malcolm, *Biopolymers*, 1983, **22**, 319.
[119] D. N. Narasing Rao, A. Kumar, H. Balaram, A. Ravi, and P. Balaram, *J. Am. Chem. Soc.*, 1983, **105**, 7423.
[120] E. K. S. Vijayakumar and P. Balaram, *Tetrahedron*, 1983, **39**, 2725.
[121] E. K. S. Vijayakumar and P. Balaram, *Biopolymers*, 1983, **22**, 2133.
[122] T. S. Sudha, E. K. S. Vijayakumar, and P. Balaram, *Int. J. Pept. Protein Res.*, 1983, **22**, 464.
[123] U. Banerjee and S. I. Chan, *Biochemistry*, 1983, **22**, 3709.
[124] H. Kimura, C. H. Stammer, Y. Shimohigoshi, C. Ren-Lin, and J. Stewart, *Biochem. Biophys. Res. Commun.*, 1983, **115**, 112.
[124a] V. J. Hruby, T. W. Rockway, V. Viswanatha, and W. Y. Chan, *Int. J. Pept. Protein Res.*, 1983, **21**, 24.
[125] P. M. Hardy and I. N. Lingham, *Int. J. Pept. Protein Res.*, 1983, **21**, 392, 406.
[126] Y. Shimohigashi, C. H. Stammer, T. Costa, and P. F. Van Voigtlander, *Int. J. Pept. Protein Res.*, 1983, **22**, 489.



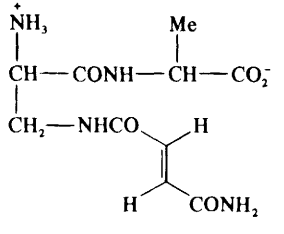
Scheme 16

N-terminal ΔTyr with a free phenolic hydroxyl.[127] [ΔLeu⁵]- and [D-Ala², ΔLeu⁵]-enkephalins, however, have been prepared free of protecting groups. The latter is almost four times as active as enkephalin in the assay using [³H]etorphine as tracer.[128] The utility of *N*-carboxy-αβ-dehydroamino acid anhydrides in the synthesis of dehydropeptides has been demonstrated. In particular a tripeptide containing a central unsaturated residue can be prepared simply, if surprisingly, in pyridine (*e.g.* Scheme 16).[129] Oxidation of the oxazolone derived from Boc-Gly-Phe-OH with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone gives, as expected, the oxazolone of Boc-Gly-ΔPhe-OH; unexpectedly, however, (60) is also formed.[130]

Structural Studies of Other Naturally Occurring Linear Peptides. – The leucine aminopeptidase inhibitor produced by *Bacillus circulans* has been identified as a dipeptide derivative, 2,3-dihydroxybenzoyl-Ala-Thr; it has no anti-microbial activity.[131] The structure of the dipeptide antibiotic A19009 from *Streptomyces collinus* has been revised from the structure proposed in 1972 (61) to the isomer (62). This reassignment has been confirmed by synthesis.[132] ^{1}H n.m.r. spectral

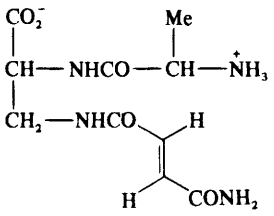


(60)

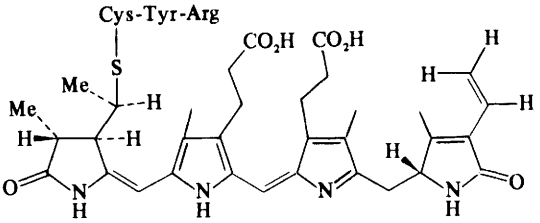


(61)

[127] Y. Shimohigashi, J. W. Dunning, jun., A. J. Kolar, and C. H. Stammer, *Int. J. Pept. Protein Res.*, 1983, **21**, 202.
[128] Y. Shimohigashi and C. H. Stammer, *J. Chem. Soc., Perkin Trans. 1*, 1983, 803.
[129] C. Shin, T. Yamada, and Y. Yonezawa, *Tetrahedron Lett.*, 1983, **24**, 2175.
[130] A. C. Bach, A. A. Baldwin, L. M. Gierasch, and A. L. Rheingold, *J. Chem. Soc., Chem. Commun.*, 1983, 1398.
[131] S. Kobaru, M. Tsunakawa, M. Hanada, M. Konishi, K. Tomita, and H. Kawaguchi, *J. Antibiot.*, 1983, **36**, 1396.
[132] J. L. van der Baan, J. W. F. K. Barnick, and F. Bickelhaupt, *J. Antibiot.*, 1983, **36**, 784.



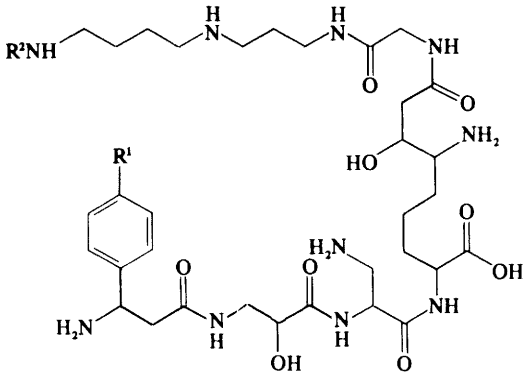
(62)



(63)

analysis of the smallest tryptic fragment derived from the α-subunit of β-phycoerythrin from *Porphyridium cruentum* has provided direct proof that the bilin is linked to the peptide through a single thioether bond (63).[133]

Edeine F from *Bacillus brevis* has been identified as (64). The edeine series of antibiotics differ only in two respects, as shown in (64). Edeine F can be prepared by amidination of Edeine D.[134] Two closely related novel antibiotics from *Metarhizium anisopliae* have proved to be the heptapeptides H-Arg-HyArg-D-Orn-Thr-D-Orn-Lys-D-Tyr-OH and H-Arg-HyArg-D-Orn-Thr-D-Orn-HyArg-D-Tyr-OH (HyArg is the unusual residue *threo*-γ-hydroxy-L-arginine).[135] The sequence



Edeine	R^1	R^2
A	OH	H
B	OH	$-CNH_2(=NH)$
D	H	H
F	H	$-CNH_2(=NH)$

(64)

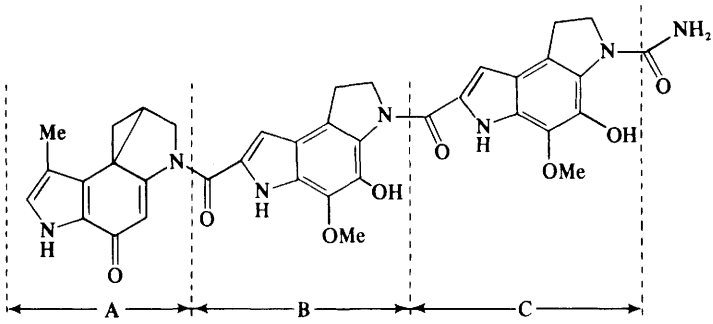
[133] R. W. Schoenleber, S.-L. Leung, D. J. Lundell, A. N. Glazer, and H. Rapaport, *J. Am. Chem. Soc.*, 1983, **105**, 4072.

[134] H. Wojciechowska, W. Zgoda, E. Borowski, K. Dziegielewski, and S. Ulikowski, *J. Antibiot.*, 1983, **36**, 793.

[135] H. Kawauchi, M. Tobno, Y. Tsuchiya, M. Hayashida, Y. Adachi, T. Mukai, I. Hayashi, S. Kimura, and S. Kondo, *Int. J. Pept. Protein Res.*, 1983, **21**, 546.

of an octapeptide lophyrotomin from the sawfly *Lophyrotoma interrupta* that acts as a liver toxin has been identified as PhCO-D-Ala-D-Phe-Val-Ile-D-Asp-Asp-D-Glu-Gln-OH. It has a hydrophobic N-terminus and a hydrophilic C-terminus. The presence of four D-residues in a peptide from such a higher organism is unprecedented, and it may not originate in the insect.[136]

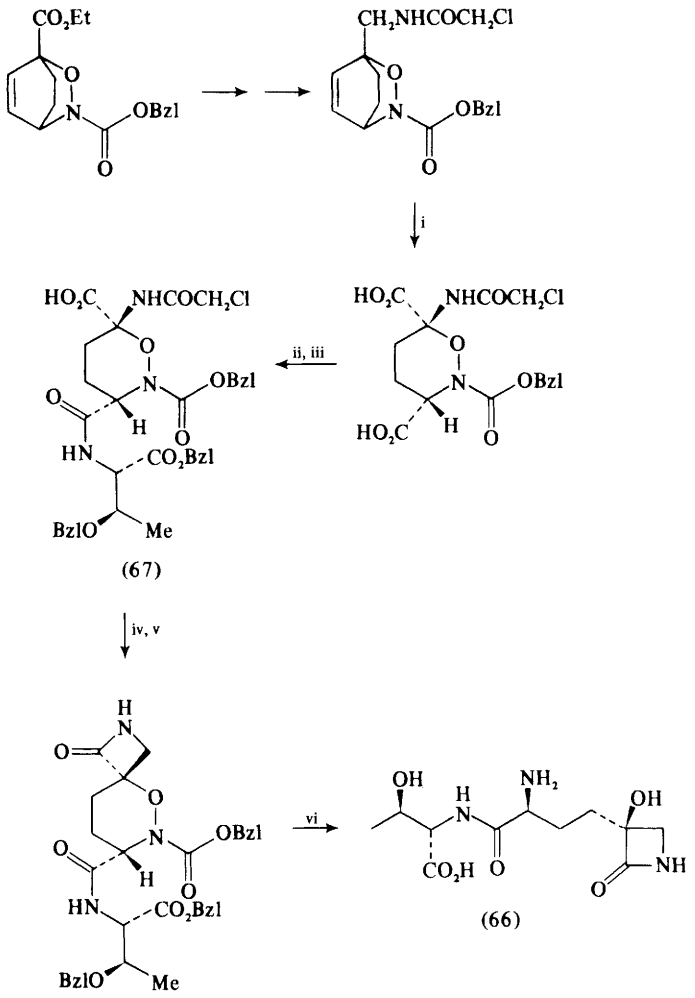
Thallium-ion-induced carbonyl-carbon chemical shifts have been compared for all of the L-residue-peptide carbonyl carbons of the Gramicidin A transmembrane channel. They indicate that this channel is a head-to-tail dimer structure composed of a left-handed single-stranded β-helix.[137] I.r. studies of this single-stranded β-helix in Gramicidin A, which has been incorporated into phospholipid structures, show an amide frequency of $1633 \pm 1\ cm^{-1}$, a frequency previously considered to be diagnostic of antiparallel double-stranded β-helix and β-sheet structures.[138] Biosynthesis of the anti-tumour antibiotic CC1065 (65) has been investigated using radiolabelled substrates. Tyrosine is a precursor of all three benzodipyrrole units, but Dopa is only incorporated into subunits B and C. Serine contributes three 2C units, with loss of C-1, to all three subunits. The *S*-Me of methionine contributes four C-1 units.[139]



(65)

Synthetic Studies of Other Linear Peptides. – The total synthesis of Edeine D (64) and decarboxy-Edeine D has been reported. The biological activity of the latter shows that the carboxyl group is not essential for the interaction of the antibiotic with its cellular target.[140] The stereospecific synthesis of tabtoxin (66), the toxin from *Pseudomonas tabaci*, which causes wildfire disease in tobacco plants, has also been accomplished (Scheme 17). The intermediate (67) was obtained as two diastereoisomers, which were separated before continuing. Tabtoxin appears to exert its effect on tobacco by a specific blockade of gluta-

[136] D. H. Williams, S. Santikarn, F. De Angelis, R. J. Smith, D. G. Reid, P. B. Oelrichs, and J. K. Macleod, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1869.
[137] D. W. Urry, T. L. Trapane, and K. U. Prasad, *Science*, 1983, **221**, 1064.
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[139] L. H. Hurley and J. S. Rokem, *J. Antibiot.*, 1983, **36**, 383.
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(67)

(66)

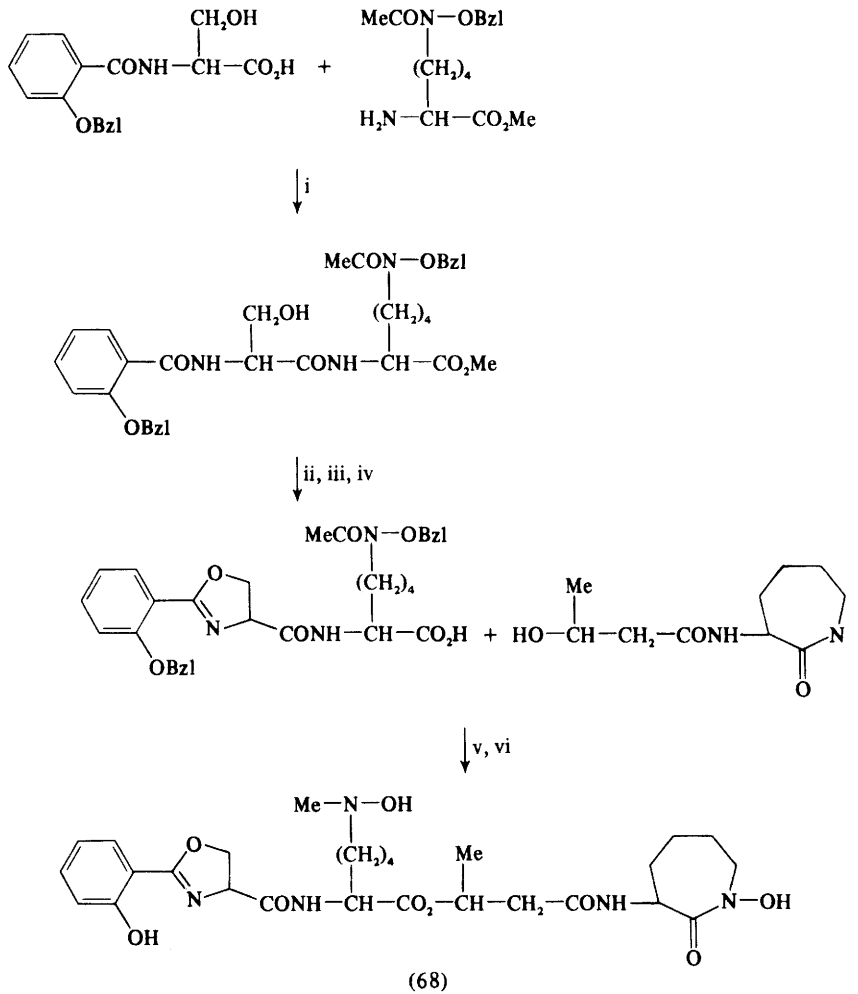
Reagents: i, $KMnO_4$, $Bu_4N^+HSO_4^-$, 25 °C; ii, Ph_3CCOCl, NEt_3; iii, Thr(OBzl) OBzl; iv, thiourea; v, 2-thiopyridinedisulphide, Ph_3P; vi, Pd, H_2

Scheme 17

mine synthetase.[141] A third novel synthesis is that of mycobactin S2 (68), and the later stages are shown in Scheme 18. The final coupling to give protected mycobactin S2 resulted in inversion at the hydroxyl group giving material of the natural *S*,*S*,*S*,*S*-configuration. The fragment (69) was prepared but could not be induced to couple with amines.[142]

[141] J. E. Baldwin, P. D. Bailey, G. Gallacher, K. A. Singleton, and P. M. Wallace, *J. Chem. Soc., Chem. Commun.*, 1983, 1049.

[142] P. J. Maurer and M. J. Miller, *J. Am. Chem. Soc.*, 1983, **105**, 240.



(68)

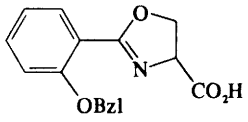
Reagents: i, EEDQ; ii, $SOCl_2$; iii, OH^-, H_2O; iv, H^+; v, PPh_3, diethyl azadicarboxylate; vi, Pd, H_2

Scheme 18

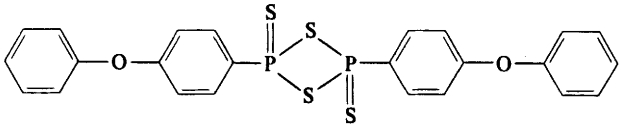
The slightly sweet thioaspartame has been made using Lawessons reagent.[143] Conversion of N-protected dipeptides into the corresponding endothiopeptides with this reagent has been described by another group,[144] and a modified Lawessons reagent (70) of higher solubility in organic solvents has also been examined. The size of amino acid side chains markedly affects the rate of thiona-

[143] B. Yde, I. Thomsen, M. Thorsen, K. Clausen, and S.-O. Lawesson, *Tetrahedron*, 1983, 4121.

[144] D. W. Brown, M. M. Campbell, and C. V. Walker, *Tetrahedron*, 1983, **39**, 1075.



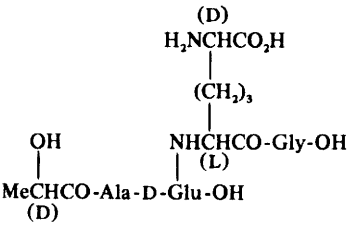
(69)



(70)

tion. In di- and tri-peptides it was found that (70) will, for example, selectively convert Boc-Phe-Gly-Pro-OMe into Boc-Phe(*S*)-Gly-Pro-OMe. Attempted elongation of thiodipeptides from the carboxyl end was frustrated by the formation of thiaoxazolinones, which proved resistant to aminolysis under standard coupling conditions.[145] *X*-Ray analysis of Z-Gly(*S*)-Gly-OBzl shows that, apart from the CO and CS bond lengths, there is no significant difference between the amide and thioamide bonds.[146]

A biotin-containing pentapeptide Boc-Glu-Ala-Met-Bct-Met (Bct = N^{ϵ}-biotinyl-L-lysine), which (apart from the N-terminal residue) corresponds to the coenzyme binding site of *E. coli* acetyl-CoA carboxylase, has been prepared. The glutamic acid residue was added to improve solubility. The synthetic peptide bound to the enzyme biotin-carboxylase subunit by one order of magnitude more tightly than biotin, but the subsequent catalytic process was less favourable than for free biotin.[147] Four analogues of the bitter heptapeptide Arg-Gly-Pro-Pro-Phe-Ile-Val isolated from cow-milk casein after treatment with the alkaline protease of *Bacillus subtilis* have been synthesized and tasted. The [desPro^3]-heptapeptide was equally as bitter as the natural material, but the [D-Pro^3], [D-Pro^4], and [D-Pro^{3,4}] analogues were markedly less bitter.[148]



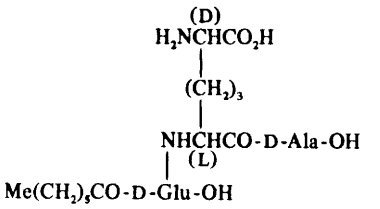
(71)

[145] G. Latoie, F. Lé Pine, L. Maziak. and B. Belleau, *Tetrahedron Lett.*, 1983, **24**, 3815.

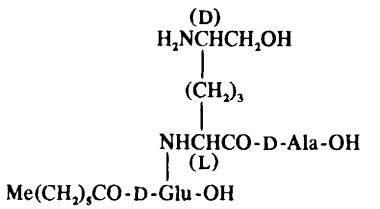
[146] T. F. M. La Cour, H. A. S. Hansen, K. Clausen, and S.-O. Lawesson, *Int. J. Pept. Protein Res.*, 1983, **22**, 509.

[147] H. Kondo, S. Uno, F. Mariuchi, J. Sunamoto, S. Ogushi, and D. Tsuru, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1176.

[148] I. Miyake, K. Konge, H. Kanehisa, and H. Okai, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1678.



(72)



(73)

Of twenty-one analogues of the immunostimulant tetrapeptide FK-156 tested for the suppression of tumour growth, six were effective. Three (71–73) were effective even when administered subcutaneously in sites remote from the tumour.[149] A careful study has been made of the preparation of the DLLL, DLDL, DLDD, and DLLD diastereoisomers of Boc-Ala-MeLeu-MeLeu-MeVal-OH. This tetrapeptide is part of the peptide sequence of cyclosporin, and the work was aimed at finding conditions conducive to minimal racemization of coupling *N*-methylamino-acids. The mixed pivalic anhydride method of carboxyl activation was found to be strongly dependent on temperature. At −20 °C, the DLL-tripeptide gave the DLLL-tetrapeptide, but at +20 °C the DLDL isomer was obtained.[150] The synthesis of four Gramicidin A1 (GA) molecules labelled with ^{13}C at the 1-position of tryptophan residues 9, 11, 13, and 15, respectively, has been reported, together with a preparation of [D-Leu^2]-GA. These compounds have been used to demonstrate that GA forms an LH helix and to probe the basis of monovalent *versus* divalent cation specificity.[151] Other synthetic work on linear peptides with structural features not typical of proteins is outlined in Table 4.[152–177]

[149] S. Izumi, K. Nakahara, T. Gotoh, S. Hashimoto, T. Kin, M. Okuhara, H. Aoki, and H. Imanaka, *J. Antibiot.*, 1983, **36**, 566.
[150] R. M. Wenger, *Helv. Chim. Acta*, 1983, **66**, 2672.
[151] D. W. Urry, T. L. Trapane, S. Romanowski, R. J. Bradley, and K. U. Prasad, *Int. J. Pept. Protein Res.*, 1983, **21**, 16.
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[153] P. A. Bartlett and C. K. Marlowe, *Biochemistry*, 1983, **22**, 4618.
[154] R. B. Harris, P. D. M. Strong, and I. B. Wilson, *Biochem. Biophys. Res. Commun.*, 1983, **116**, 394.
[155] K.-S. Cheung, S. A. Wasserman, E. Dudek, S. A. Lerner, and M. Johnston, *J. Med. Chem.*, 1983, **26**, 1733.
[156] C. D'Silva and K. T. Douglas, *J. Org. Chem.*, 1983, **48**, 263.
[157] R. E. Galardy, V. Kontoyiannidon-Ostrem, and Z. P. Kartylewicz, *Biochemistry*, 1983, **22**, 1990.
[158] P. Kafarski, B. Lejczak, P. Mastalerz, J. Szewezyk, and C. Wasielewski, *Can. J. Chem.*, 1982, **60**, 3081.
[159] M. Ballaster, J. Riera, J. Costaner, C. Rovira, J. Veciana, and C. Ourubia, *J. Org. Chem.*, 1983, **48**, 3716.
[160] M. Kawai, K. Kuwabara, R. Kimura, and S. Sekido, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 347.
[161] F. H. C. Stewart, *Aust. J. Chem.*, 1983, **36**, 1629.
[162] F. H. C. Stewart, *Aust. J. Chem.*, 1983, **36**, 2511.
[163] O. A. Thompson, A. I. Scott, and R. L. Baxter, *J. Chem. Soc., Perkin Trans. 1*, 1983, 941.

Table 4 *Linear peptides not discussed in the text*

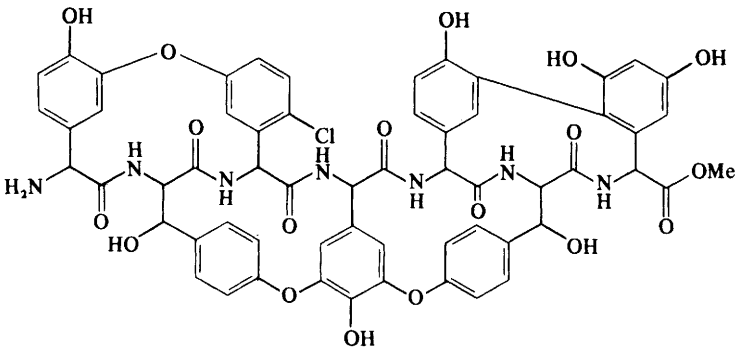
Compound	*Aim*	*Ref.*
Bestatin	New synthesis	152
Z-NHCH$_2$P(O$_2^-$)-Leu-Xxx-OH	Thermolysin inhibitors	153
Dipeptide hydroxamates	Angiotensin-converting enzyme inhibitors	154
β-ClAla and propargyl glycine dipeptides	Suicide substrates	155
PhCO-Phe(2,3-OH)-Gly-OH	Dopamine β-hydroxylase binding	156
N-Phosphoryl-Ala-Pro-H	Angiotensin-converting enzyme inhibitor	157
Phosphonodipeptides	Synthetic method	158
Spin-labelled di- and tri-peptides		159
Di- to tetra-peptide derivatives of hesperitin dihydrochalcone	Structure–taste relation	160
p-Aminobenzoic acid peptide	Enkephalin analogue	161
m-Aminomethylbenzoic acid peptide	Enkephalin analogue	162
δ-(α-Aminoadipyl)-Cys-D-Val-OH	New synthesis	163
Dnp-Leu-Xxx-Pro-Val-*p*-nitroanilides	Conformational study	164
Doxarubicin tripeptide	Plasma substrate	165
Bialaphos	Herbicide	166
Pepstatin analogues		167
Tyr-analogue peptide	Enkephalin analogue	168
3-Fluoro-Ala-peptides	Antibody sites	169
Keto analogue of pepstatin		170
β-Cyclopropyl-Ala analogues	Enkephalin analogue	171
1-Aminoalkanephosphonic acid analogues of Leu and Met	Enkephalin analogue	172
trans-4-Hydroxycinnamic analogue of Tyr	Enkephalin analogue	173
β-Pyrazinyl-L-Ala analogue of Phe	Enkephalin analogue	174
Dnp-Gly$_{2 \text{ or } 3}$-D-Ala-Pro-Gly$_{2 \text{ or } 3}$-*p*-nitroanilides	Conformational study	175
[Sar^1,Phe(3′,5′-Br$_2$-4′-Cl)^8]- and [Sar^1,Phe-(2′,3′,4′,5′,6′-Br$_5$)^8]-angiotensins		176
Boc-(Val-D-Val)$_6$-OMe	Conformational study	177
[Nle^{15}]- and [methoxinine^{15}]-human little gastrin I		178

[164] T. Higashima, K. Sato, and U. Nagai, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1527, 1657, 3323.
[165] P. K. Chakravarty, P. L. Carl, M. J. Weber, and J. A. Katzenellenbogen, *J. Med. Chem.*, 1983, **26**, 638.
[166] H. Seto, S. Imai, T. Tsuruoka, A. Satoh, M. Kojima, S. Inouye, T. Sasaki, and N. Otake, *J. Antibiot.*, 1982, **35**, 1719.
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[168] T. Deeks, P. A. Crooks, and R. D. Waigh, *J. Med. Chem.*, 1983, **26**, 762.
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[170] C. H. Rich and M. S. Bernatowicz, *J. Org. Chem.*, 1983, **48**, 1999.
[171] N. Muthukumaraswamy, A. R. Day, D. Pinon, C. S. Liao, and R. J. Freer, *Int. J. Pept. Protein Res.*, 1983, **22**, 305.
[172] L. Kupczyk-Subotowska and P. Mastalerz, *Int. J. Pept. Protein Res.*, 1983, **21**, 485.
[173] C. Amar, E. Vilkas, S. Laurent, B. Gautray, and H. Schmitt, *Int. J. Pept. Protein Res.*, 1983, **22**, 434.

6 Glycopeptides

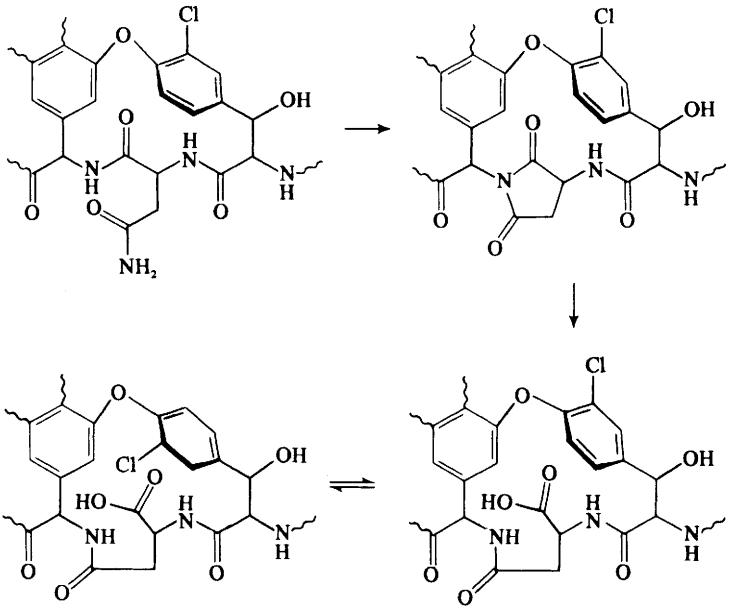
Glycopeptide Antibiotics. – The structure of component A35512B of the glycopeptide antibiotics from *Streptomyces candidus* (74) has been confirmed with respect to the position of the chlorine atom and the absolute configuration. This compound has an essentially identical peptide sequence, absolute configuration, and conformation to ristocetin.[179] Vancomycin has been shown to contain asparagine rather than isoasparagine as proposed previously. Reduction of vancomycin aglycone (after diazomethane treatment to protect the phenolic hydroxyl group) with diborane in THF then acid hydrolysis gives 2,4-diaminobutyric acid and no 3,4-diaminobutyric acid. Vancomycin is unique in its group in containing asparagine.[180] A mechanism has been proposed for the rearrangement of vancomycin at pH 4 to CDP-I (Scheme 19); the Cl-substituted ring may rotate in CDP-I, whereas in vancomycin itself the ring system is smaller and does not allow rotation.[180] In the biosynthesis of ristocetin by *Nocardia lurida* it has been found that *m*,*m*′-dihydroxyphenylglycine and *p*-methyl-*m*,*m*′-dihydroxyphenylglycine can be derived from acetic acid, whereas *p*-hydroxyphenylglycine and β-hydroxytyrosine are derived from tyrosine. A mode of acetate incorporation consistent with the observed labelling pattern is suggested (Scheme 20).[181]

Other Glycopeptides. – Two glycosylated analogues of substance P, β-D-Glcp-(1 → 5)-$Gln_{2\,or\,3}$-Phe-Phe-Gly-Leu-Met-NH_2, have been prepared to try and increase the poor solubility of the active C-terminal fragment of substance P. The products did prove appreciably more soluble, and the bioactivity and half-life

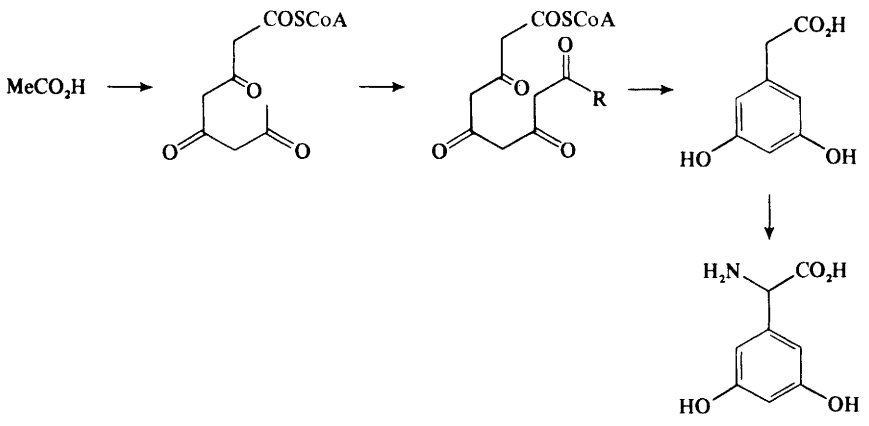


(74)

[174] C. Petermann and J.-L. Fauchère, *Helv. Chim. Acta*, 1983, **66**, 1513.
[175] K. Sato, N. Taki, U. Nagai, and T. Higashijima, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2476.
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[179] C. M. Harris and T. M. Harris, *Tetrahedron*, 1983, **39**, 1661.
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[181] S. J. Hammond, D. H. Williams, and R. V. Nielson, *J. Chem. Soc., Chem. Commun.*, 1983, 116.



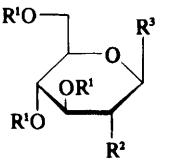
Scheme 19



Scheme 20

are only slightly modified.[182] The G1–G5 sequence of human glycophorin A^M, H-Ser^1-Ser^2-Thr^3-Thr^4-Gly-OH, with 2-acetamido-2-deoxy-α-d-galactopyranosyl residues attached to the hydroxyls of residues 2, 3, and 4, has been synthesized. A useful n.m.r. analysis of this peptide derivative proved possible. Residues 2–4

[182] C. Poujade, S. Lavielle, Y. Tarrens, and A. Marquet, *Int. J. Pept. Protein Res.*, 1983, **21**, 254.



		R^1	R^2	R^3
(75)	a;	Bzl	OBzl	Fmoc-Asn-Leu-Ser-OBzl
	b;	Ac	NHAc	Peoc-Pro-Ile-Asn-OBu^t
	c;	Ac	NHAc	Peoc-Met-Ser-Asn-OBzl
	d;	Ac	NHAc	Z-Asn-Leu-Ser-OMe
	e;	Bzl	OBzl	Z-Ser-Ala-Gly-OCH_2CH_2Br
	f;	Bzl	OBzl	Z-Ser-Val-Phe-OCH_2CH_2Br
	g;	Bzl	OBzl	Z-Ser-Leu-Phe-OCH_2CH_2Br
	h;	Bzl	OBzl	Z-Ser-Phe-Val-OCH_2CH_2Br

were added stepwise as *N*-Fmoc *O*-glycosyl derivatives.[183] A tris-glycosylated derivative of the glycophorin A fragment Ser-Thr-Thr has been independently prepared.[184] Fmoc protection has also proved useful in the synthesis of an *O*-glycosyl tripeptide (75a).[185] Protected glycotripeptides corresponding to partial sequences of bovine deoxyribonuclease A (75b) and luteinizing hormone (75c) have been prepared using EEDQ couplings,[186] and the glycotripeptide (75d) has been synthesized in a similar way as a model for the $O \rightarrow N$ glycosyl shift.[187] Another series of glycotripeptides (75e–h) have been prepared by a stepwise synthesis from the N-terminus, using 2-bromoethyl esters for C-protection. Removal of these was effected by prior conversion to the iodoethyl esters before cleavage using zinc.[188] Glycosylation of a suitably protected threonine-containing peptide has been found to be a less successful synthetic method than a stepwise strategy to incorporate *O*-galactosylthreonine.[189]

Natural-abundance ^{13}C n.m.r. spectra of α- and β-D-galactopyranosyl-L-Thr, D-Thr, L-*allo*-Thr, and D-*allo*-Thr show distinct anomeric (C1) and Thr methyl (C'γ) signals that enable the enantiomeric purity of synthetic *O*-glycopeptides

[183] A. A. Pavia and B. Ferrari, *Int. J. Pept. Protein Res.*, 1983, **22**, 539, 549.
[184] V. V. Bencomo and B. Sinay, *Carbohydr. Res.*, 1983, **116**, C9.
[185] P. Schultheiss-Reimann and H. Kunz, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 62.
[186] H. Kunz and H. Kauth, *Liebigs Ann. Chem.*, 1983, 337.
[187] H. Kauth and H. Kunz, *Liebigs Ann. Chem.*, 1983, 360.
[188] M. Buchholz and H. Kunz, *Liebigs Ann. Chem.*, 1983, 1859.
[189] J. M. Lacombe and A. A. Pavia, *J. Org. Chem.*, 1983, **48**, 2557.

to be monitored.[190] ^{13}C n.m.r. studies of two *O*-D-galactosylated tripeptides of Thr and Gly,[191] of the interactions of Gd^{III} with mono-*O*-glycosylated tripeptides,[192] and of Gd^{III} and Mn^{II} with two vicinally di-*O*-D-galactosylated tripeptides[193] have been reported.

1-*O*-Acetyl, decanoyl, and hexadecanoyl derivatives of muramyl dipeptide (MDP) all show strong immunoadjuvant activity, showing that lipophilicity of C-1 does not diminish potency.[194] *N*-Acetyl-1-thiomuramyl-Ala-D-iGln has an activity comparable to that of MDP,[195] as does MDP bonded to the fluorescent adduct 2-(fluoresceinylamino)-4,6-dichloro-*s*-triazine through the 6-position of the sugar.[196] The solid-phase synthesis of *N*-Ac-muramyl-Abu-D-iGln-$(Lys)_3$-OH has been reported. In aqueous solution it exists as a mixture of α- and β-anomers, and its immunoadjuvant activity is comparable with that of *N*-Ac-muramyl-Abu-D-iGln-OH.[197] The 400 MHz ^1H n.m.r. spectrum of MDP in DMSO shows the same α : β anomeric ratio (2 : 1) as reported for the equilibrated aqueous solutions from which the DMSO solution was prepared.[198]

[190] A. A. Pavia and J. M. Lacombe, *J. Org. Chem.*, 1983, **48**, 2564.
[191] K. Dill, R. E. Hardy, J. M. Lacombe, and A. A. Pavia, *Carbohydr. Res.*, 1983, **114**, 147.
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[196] C. K. Hilbert, W. C. Kopp, H. B. Richerson, and C. F. Barfknecht, *J. Med. Chem.*, 1983, **26**, 1729.
[197] V. Krchnák, J. Jezek, and M. Zaoral, *Collect. Czech. Chem. Commun.*, 1983, **48**, 2079.
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Appendix

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IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN)

Nomenclature and Symbolism for Amino Acids and Peptides

Recommendations 1983

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These are recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), whose members are H.B.F.Dixon (chairman), A. Cornish-Bowden (secretary), C. Liébecq (as chairman of the IUB Committee of Editors of Biochemical Journals), K. L. Loening, G. P. Moss, J. Reedijk, S.F. Velick, and J. F. G. Vliegenthart. Comments may be sent to any member of the commission, or to its secretary: A. Cornish-Bowden, Department of Biochemistry, University of Birmingham, P. O. Box 363, Birmingham, England, B15 2TT. JCBN thanks many who helped with drawing up the recommendations, especially P. Karlson, its former chairman, B. Keil, a former member of the Nomenclature Committee of IUB (NC–IUB), other members and former members of NC–IUB, namely H. Bielka, N. Sharon and E. C. Webb, and also W. E. Cohn, J. T. Edsall, J. S. Morley, G. T. Young, and members of the IUPAC Commission on Nomenclature of Organic Chemistry (CNOC).

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INTRODUCTION

The traditional and well-known names of the common α-amino acids were, in general, given to them by their discoverers and bear no relationship to their chemical structures [1, 2]. The modification of these names to accommodate derivatives and to designate configuration was codified in 1947 [3] and revised in 1960 [4]. After proposals for the revision of the rules for naming α-amino acids with two centres of chirality had appeared in 1963 [5], a complete revision of the rules was made in 1974 [6] on the basis of a report by a committee convened by H. B. Vickery. Recommendations for symbols for amino-acid residues in peptide sequences made by Brand & Edsall (p. 224 in [8]) were revised in 1966 [9] and 1971 [10], and recommendations for a one-letter notation were approved in 1968 [11]. Recommendations for naming and symbolizing sequences derived from those of named peptides were made in 1966 [12].

The present revision combines all these documents. In Part 1 on *nomenclature*, the main changes are to propose names for particular ionic forms of residues (3AA-12.2) and to apply the stereochemical rules [13] more fully (3AA-3). Part 2 on *symbolism* introduces a few new symbols (3AA-15.2.7), simplifies the designation of ionized forms of peptides (3AA-19.3), explains the principles for giving symbols to reagents (3AA-18.2), presents a method for showing how parts of residues react (3AA-17.4), and describes the one-letter system for representing long sequences (3AA-20 and -21). Part 3, on the *modification of named peptides*, is extended to cover enantiomers and reversed sequences (3AA-22.7) and peptide analogues (3AA-22.8). Symbols for the twenty ribosomally incorporated (coded) amino acids are given in Table 1, and symbols used in these recommendations for other amino acids are mostly listed in the Appendix, although a few others are given in 3AA-15.2. Substantially new recommendations are marked by triangles in the margins.

Part 1. **Nomenclature**

Part 1, Section A: AMINO-ACID NOMENCLATURE

3AA-1. NAMES OF COMMON α-AMINO ACIDS

The trivial names of the α-amino acids that are commonly found in proteins and are represented in the genetic code, together with their symbols, systematic names [14] and formulas, are given in Table 1. Some other common amino acids are listed in the Appendix.

When the phrase 'amino acid' is a qualified noun it contains no hyphen; a hyphen is inserted when it becomes an adjective so as to join its components in qualifying another noun, e.g. amino-acid sequence.

3AA-2. FORMATION OF SEMISYSTEMATIC NAMES FOR AMINO ACIDS AND DERIVATIVES

3AA-2.1. *Principles of Forming Names*

Semisystematic names of substituted α-amino acids are formed according to the general principles of organic nomenclature [14], by attaching the name of the substituent group to the trivial name of the amino acid. The position of the substitution is indicated by locants (see 3AA-2.2). The configuration, if known, should be indicated (see 3AA-3, 3AA-4).

New trivial names should not be coined for newly discovered α-amino acids unless there are compelling reasons. When they are needed (e.g. because the substance is important and its semisystematic name is cumbersome), the name should be constructed according to the general principles for naming natural products [15], including either some element of its chemical structure or reference to its biological origin. It is important to use no elements in the trivial name that imply an incorrect structure; when a new trivial name is used, it is essential that it be defined by a correctly constructed systematic or semisystematic name.

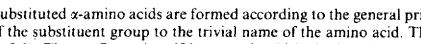
A number of existing trivial names are given in the Appendix, and an extensive list has been published previously [6].

3AA-2.2 *Designation of Locants*

Note. The atom numbering given below is the normal chemical system for designating locants. A somewhat different system has been recommended for describing polypeptide conformations [16], in which Greek letters are used irrespective of the nature of the atom (unless it is hydrogen), so that in lysine N-6 becomes N^{ε}, and in phenylalanine C-1, C-2 and C-6 become C^{γ}, $C^{\delta 1}$ and $C^{\delta 2}$ respectively.

2.2.1. Acyclic Amino Acids

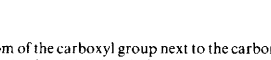
In acyclic amino acids, the carbon atom of the carboxyl group next to the carbon atom carrying the amino group is numbered 1. Alternatively, Greek letters may be used, with C-2 being designated α. This practice is not encouraged for locants, although terms like 'α-amino acids' and 'α-carbon atom' are retained. Example:



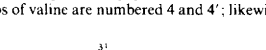
lysine

A heteroatom has the same number as the carbon atom to which it is attached, e.g. N-2 is on C-2. When such numerals are used as locants they may be written as N^{6}- or as 6-*N*, e.g. N^{6}-acetyllysine.

▲ The carbon atoms of the methyl groups of valine are numbered 4 and 4′; likewise those of leucine are 5 and 5′. Isoleucine is numbered as follows:

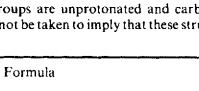
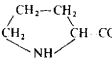
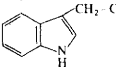
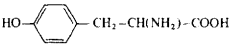


The word 'methyl' can be italicized for use as a locant for substitution on (or isotopic modification {Section H in [14]} of) the ▲ methyl group of methionine, e.g. [*methyl*-^{14}C]methionine. The nitrogen atoms of arginine are designated as shown for the arginine (1 +)cation:



It should be noted that the ω and ω′ atoms of this cation are equivalent because of resonance. The carbon atom in the guanidino group may be called guanidino–C (it may be needed as a locant for isotopic replacement although it cannot carry a substituent).

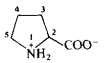
Table 1. *α-Amino acids incorporated into protein under mRNA direction*
The systematic names and formulas given refer to hypothetical forms in which amino groups are unprotonated and carboxyl groups are undissociated. This convention is useful to avoid various nomenclatural problems but should not be taken to imply that these structures represent an appreciable fraction of the amino-acid molecules.

Trivial name[a]	Symbol	One-letter symbol[b]	Systematic name[c]	Formula
Alanine	Ala	A	2-Aminopropanoic acid	CH_3–$CH(NH_2)$–COOH
Arginine	Arg	R	2-Amino-5-guanidinopentanoic acid	H_2N–$C(=NH)$–NH–$[CH_2]_3$–$CH(NH_2)$–COOH
Asparagine	Asn[d]	N[d]	2-Amino-3-carbamoylpropanoic acid	H_2N–CO–CH_2–$CH(NH_2)$–COOH
Aspartic acid	Asp[d]	D[d]	2-Aminobutanedioic acid	HOOC–CH_2–$CH(NH_2)$–COOH
Cysteine	Cys	C	2-Amino-3-mercaptopropanoic acid	HS–CH_2–$CH(NH_2)$–COOH
Glutamine	Gln[d]	Q[d]	2-Amino-4-carbamoylbutanoic acid	H_2N–CO–$[CH_2]_2$–$CH(NH_2)$–COOH
Glutamic acid	Glu[d]	E[d]	2-Aminopentanedioic acid	HOOC–$[CH_2]_2$–$CH(NH_2)$–COOH
Glycine	Gly	G	Aminoethanoic acid	$CH_2(NH_2)$–COOH
Histidine	His	H	2-Amino-3-(1*H*-imidazol-4-yl)propanoic acid	
Isoleucine	Ile	I	2-Amino-3-methylpentanoic acid[e]	C_2H_5–$CH(CH_3)$–$CH(NH_2)$–COOH
Leucine	Leu	L	2-Amino-4-methylpentanoic acid	$(CH_3)_2CH$–CH_2–$CH(NH_2)$–COOH
Lysine	Lys	K	2,6-Diaminohexanoic acid	H_2N–$[CH_2]_4$–$CH(NH_2)$–COOH
Methionine	Met	M	2-Amino-4-(methylthio)butanoic acid	CH_3–S–$[CH_2]_2$–$CH(NH_2)$–COOH
Phenylalanine	Phe	F	2-Amino-3-phenylpropanoic acid	C_6H_5–CH_2–$CH(NH_2)$–COOH
Proline	Pro	P	Pyrrolidine-2-carboxylic acid	
Serine	Ser	S	2-Amino-3-hydroxypropanoic acid	HO–CH_2–$CH(NH_2)$–COOH
Threonine	Thr	T	2-Amino-3-hydroxybutanoic acid[e]	CH_3–$CH(OH)$–$CH(NH_2)$–COOH
Tryptophan	Trp	W	2-Amino-3-(1*H*-indol-3-yl)propanoic acid	
Tyrosine	Tyr	Y	2-Amino-3-(4-hydroxyphenyl)propanoic acid	
Valine	Val	V	2-Amino-3-methylbutanoic acid	$(CH_3)_2CH$–$CH(NH_2)$–COOH
▲ Unspecified amino acid	Xaa	X		

[a] The trivial name refers to the L or D or DL-amino acid; for those that are chiral only the L-amino acid is used for protein biosynthesis.
[b] Use of the one-letter symbols should be restricted to the comparison of long sequences (3AA-20).
[c] The fully systematic forms ethanoic, propanoic, butanoic and pentanoic may alternatively be called acetic, propionic, butyric and valeric, respectively. Similarly, butanedioic ≡ succinic, 3-carbamoylpropanoic ≡ succinamic, pentanedioic ≡ glutaric, and 4-carbamoylbutanoic ≡ glutaramic.
▲ [d] The symbol Asx denotes Asp or Asn; likewise B denotes N or D. Glx and Z likewise represent glutamic acid or glutamine or a substance, such as 4-carboxyglutamic acid, Gla (3AA-15.2.6), or 5-oxoproline, Glp (3AA-16.5), that yields glutamic acid on acid hydrolysis of peptides.
[e] See 3AA-3 and -4 for stereochemical designation.

2.2.2. Proline

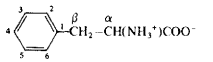
The carbon atoms in proline are numbered as in pyrrolidine, the nitrogen atom being numbered 1, and proceeding towards the carboxyl group.

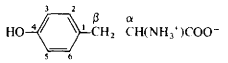


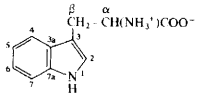
2.2.3. Aromatic Rings

The carbon atoms in the aromatic rings of phenylalanine, tyrosine and tryptophan are numbered as in systematic nomenclature, with 1 (or 3 for tryptophan) designating the carbon atom bearing the aliphatic chain. The carbon atoms of this chain are designated α (for the carbon atom attached to the amino and carboxyl groups) and β (for the atom attached to the ring system).

Note. This numbering should also be used for decarboxylated products (e.g. tryptamine).

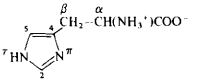

phenylalanine


tyrosine


tryptophan

2.2.4 Histidine

The nitrogen atoms of the imidazole ring of histidine are denoted by *pros* ('near', abbreviated π) and *tele* ('far', abbreviated τ) to show their position relative to the side chain. This recommendation [6, 10] arose from the fact that two different systems of numbering the atoms in the imidazole ring of histidine had both been used for a considerable time (biochemists generally numbering as 1 the nitrogen atom adjacent to the side chain, and organic chemists designating it as 3). The carbon atom between the two ring nitrogen atoms is numbered 2 (as in imidazole), and the carbon atom next to the τ nitrogen is numbered 5. The carbon atoms of the aliphatic chain are designated α and β as in 2.2.1 and 2.2.3 above. This numbering should also be used for the decarboxylation product histamine and for substituted histidine.



histidine

2.2.5. Definition of Side Chain

▲ When amino acids are combined in proteins and peptides, C-1, C-2 and N-2 of each residue (the numbering being that of aliphatic amino acids) form the repeating unit of the main chain ('backbone') and the remainder forms a 'side chain'. Hence the words 'side chain' refer to C-3 and higher numbered carbon atoms and their substituents.

3AA-2.3. *Use of the Prefix 'homo'*

An α-amino acid that is otherwise similar to one of the common ones (Table 1), but that contains one more methylene group in the carbon chain, may be named by prefixing 'homo' to the name of that common amino acid. 'Homo' in the sense of a higher homologue (F-4.5 of [15]) is commonly used for homoserine (2-amino-4-hydroxybutanoic acid) and homocysteine (2-amino-4-mercaptobutanoic acid).

3AA-2.4. *Use of the Prefix 'nor'*

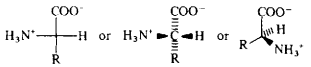
▲ The prefix 'nor' denotes removal of a methylene group (Sections F-4.2 and F-4.4 of [15]), but this is not the sense in which it has been used in the names 'norvaline' and 'norleucine'. Such names, although widely used, may therefore be misinterpreted, so we cannot recommend them, especially since the systematic names for the compounds intended, 2-aminopentanoic acid and 2-aminohexanoic acid, are short.

3AA-3. CONFIGURATION AT THE α-CARBON ATOM

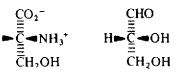
3AA-3.1. *Use of* D *and* L

The absolute configuration at the α-carbon atom of the α-amino acids is designated by the prefixed small capital letter D or L to indicate a formal relationship to D- or L-serine and thus to D- or L-glyceraldehyde. The prefix ξ (Greek xi) indicates unknown configuration.

The structures of amino acids may be drawn to show configuration in several ways [13]. In the Fischer-Rosanoff convention each chiral centre is projected onto the plane of the paper in the orientation such that the central atom appears as the point of intersection of two straight lines joining the attached groups in pairs, so that one straight line (which should be vertical) joins three atoms of the principal chain. The central atom is then considered to lie in the plane of the paper, the other atoms of the principal chain behind the plane from the viewer, and the remaining two groups in front of this plane. Thus an L-α-amino acid may be represented as



The relationship between serine and glyceraldehyde may therefore be represented as:



D-serine D-glyceraldehyde

3AA-3.2. *Position of Prefix*

In naming α-amino acids as derivatives of substances that have well-known trivial names, the prefix L or D is placed immediately before the trivial name of the parent amino acid and set off by a hyphen. Examples: *trans*-4-hydroxy-L-proline; 3,5-diiodo-L-tyrosine.

Note. Admissible exceptions to this rule are L-hydroxyproline and L-hydroxylysine, but only in general biochemical writing in a context such that the position of substitution is well understood. Note further that in the names of optically active derivatives of glycine, such as L-2-phenylglycine, the prefix must be placed before the name of the substituent as glycine itself is achiral. In the names of salts, esters and other derivatives, including peptides, the prefix is placed immediately before the trivial name of the parent acid or its radical. Examples: L-histidine monohydrochloride monohydrate; copper(II) L-aspartate; D-lysine dihydrochloride; *N*-acetyl-L-tryptophan; diethyl D-glutamate; N^6-methyl-L-lysine.

Other semisystematic names involving α-amino-acid configurations are treated similarly. Example: *S*-(D-2-amino-2-carboxyethyl)-D-homocysteine, or *S*-(D-alanin-3-yl)-D-homocysteine (3AA-8), i.e. D-cystathionine.

3AA-3.3. *Omission of Prefix*

The prefix may be omitted where the amino acid is stated to be or is obviously derived from a protein source and is therefore assumed to be L. It may also be omitted where the amino acid is synthetic and not resolved and is therefore, save in exceptional cases, an equimolecular mixture of the enantiomers. Likewise it may be omitted in a general statement that is true for either enantiomer or for any mixture of these.

3AA-3.4. *Subscripts to* D *and* L

Where confusion is possible between the use of the small capital letter prefix for the configuration of the α-carbon atom in amino-acid nomenclature and for that of the highest numbered chiral carbon atom in carbohydrate nomenclature [17], a subscript (lower case Roman letter) is added to the small capital letter prefix. If the prefix is used in the amino-acid sense, the subscript is s (for serine); if the prefix is used in the carbohydrate sense, the subscript is g (for glyceraldehyde).

Examples: L$_s$-threonine, for which the synonym in carbohydrate nomenclature is 2-amino-2,4-dideoxy-D$_g$-threonic acid; D$_s$-threonine, for which the synonym is 2-amino-2,4-dideoxy-L$_g$-threonic acid; L$_s$-allothreonine, for which the synonym is 2-amino-2,4-dideoxy-L$_g$-erythronic acid; D$_s$-allothreonine, for which the synonym is 2-amino-2,4- dideoxy-D$_g$-erythronic acid.

Note that the subscripts are essential only in discussions where both amino-acid names and those of carbohydrate derivatives occur. Nevertheless, these subscripts are highly desirable if D or L is used in naming α-amino acids that possess more than one centre of chirality (see 3AA-4).

3AA-3.5. *The* RS *System*

A more general system of stereochemical designation, which is especially convenient when there is no simple way of relating a compound to a defined standard, is the *RS* system of Cahn, Ingold & Prelog [13, 18]. In this system the ligands of a chiral atom are placed in an order of preference, based largely on atomic number. If the first three ligands appear clockwise in this order when viewed from the side remote from the least-preferred (fourth) ligand, the chiral centre is *R*; if anticlockwise, it is *S*.

The L-configuration, possessed by the chiral α-amino acids found in proteins, nearly always corresponds to *S* in the *RS* system. The most important exceptions are L-cysteine and L-cystine (see Appendix), which are *R* (in most amino acids the order of preference of the groups around C-2 is NH_3^+, COO^-, R, H, but in cysteine and cystine the group R takes precedence over carboxylate because the atomic number of sulfur attached to C-3 is higher than that of oxygen attached to C-1).

3AA-3.6. *Amino Acids Derived from Amino Sugars*

Amino acids that are derived from amino sugars and contain five or more carbon atoms are named in conformity with the system of carbohydrate nomenclature [17] or with a recommended trivial name.

Examples: (1) D$_g$-glucosaminic acid for 2-amino-2-deoxy-D$_g$-gluconic acid, the α-carbon of which has the configuration of that in D-serine, and in which C-5, the highest numbered chiral centre, also has the D-configuration; (2) D$_g$-mannosaminic acid for 2-amino-2-deoxy-D$_g$-mannonic acid, the α-carbon of which has the configuration of that in L-serine, but in which C-5 has the D configuration. The subscript g may be omitted unless confusion with the amino-acid use of the designations D and L is likely.

3AA-3.7. *Use of* meso

The prefix *meso-*, in lower case italic letters, is used to denote those amino acids or derivatives that, although they contain chiral groups, are achiral, usually because of a plane of symmetry, e.g. *meso*-lanthionine.

3AA-3.8. *Use of* DL

A mixture of equimolar amounts of D and L compounds is termed racemic and is designated by the prefix DL (no comma), e.g. DL-leucine. It may alternatively be designated by the prefix *rac-* (e.g. *rac*-leucine) or by the prefix (±)- (see 3AA-5).

3AA-4. CONFIGURATION AT CHIRAL CENTRES OTHER THAN THE α-CARBON

3AA-4.1. *The Sequence Rule*

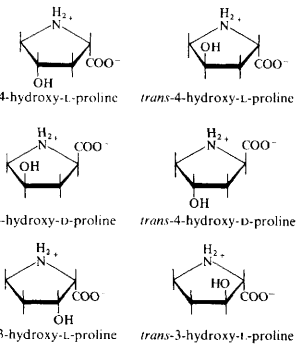
The *RS* system (3AA-3.5) is preferred for designating configuration at centres other than α-C, e.g. (2*S*,3*R*)-threonine. To avoid using two different systems of designation in the same name, (2*S*,4*S*)-4-hydroxyproline may be used instead of (4*S*)-4-hydroxy-L-proline.

3AA-4.2 *Carbohydrate Prefixes*

▲ The use of carbohydrate prefixes (e.g., D-*erythro*) cited in the 1974 version of these recommendations [6] as an alternative system for α-amino acids having two or more chiral centres is now discouraged.

3AA-4.3. *Use of* cis *and* trans

The amino acids 4-hydroxy-L-proline and 3-hydroxy-L-proline and analogous substituted prolines may also be named as follows (cf. 3AA-3.2).



cis-4-hydroxy-L-proline *trans*-4-hydroxy-L-proline

cis-4-hydroxy-D-proline *trans*-4-hydroxy-D-proline

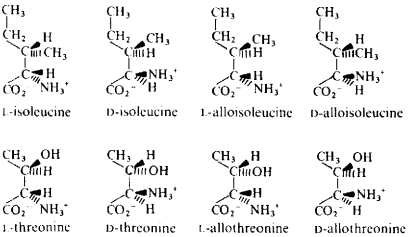
cis-3-hydroxy-L-proline *trans*-3-hydroxy-L-proline

The prefixes *cis* and *trans* refer to the relative positions of the hydroxyl and carboxyl groups in each compound.

Comment. The hydroxyprolines found in collagen are *trans*-4-hydroxyproline (predominantly) and *trans*-3-hydroxyproline. The prefixes may be omitted when no ambiguity arises (cf. 3AA-3.3).

3AA-4.4. *Use of 'allo'*

Amino acids with two chiral centres were named in the past by allotting a name to the first diastereoisomer to be discovered. The second diastereoisomer, when found or synthesized, was then assigned the same name but with the prefix allo-. This method ▲ can be used only with trivial names (see 2.1) but not with semisystematic or systematic names. It is now recommended that allo should be used only for alloisoleucine and allothreonine, as follows:



3AA-4.5. *Designation of Centres with Unknown Configurations*

▲ When absolute or relative configurations at one or more centres are not known, such designations as 'isomer A' and 'isomer B' are frequently employed until the full configurational relationships are established.

▲ If the configuration is known at one centre but not at a second, the *RS* system is used for the known centre, with a Greek xi (ξ), meaning 'unknown configuration' for the other, e.g. (2*S*,5ξ)-2-amino-5-hydroxyhexanoic acid (a single stereoisomer). If the configuration at two centres is unknown, the ξ may be used as in the example (2ξ,5ξ)-2-amino-5-hydroxyhexanoic acid. If a racemate is to be designated, this is done by reference to its optical activity (3AA-5), e.g. (±)-(2ξ,5ξ)-2-amino-5-hydroxyhexanoic acid. If the relative configuration of two centres is known, but the absolute is unknown, '*R**' and '*S**' may be used, e.g. (2*R**,5*S**)-2-amino-5-hydroxyhexanoic acid.

3AA-4.6. *Other Stereochemical Features*

When other stereochemical elements are encountered, such as *E*/*Z* double-bond isomers, they are described according to the provisions of Section E of the IUPAC rules for organic nomenclature [13].

3AA-5. OPTICAL ROTATION

If it is desired to indicate the direction of rotation of plane polarized light of specified wavelength in a specified solvent, this can be done with a 'plus' or 'minus' sign in parenthesis (E-4.4 of reference [13]), e.g. (+)-6-hydroxytryptophan. This may be particularly useful if the configuration at C-2 is not known, but it may also be done for emphasis, with or without a configurational symbol D or L, when this configuration is known, e.g. (+)-glutamic acid, or (+)-L-glutamic acid. A racemic amino acid (3AA-3.8) may be indicated by (±), e.g. (±)-leucine.

Part 1, Section B: NOMENCLATURE OF NON-PEPTIDE DERIVATIVES OF AMINO ACIDS

If an amino acid is substituted on a saturated carbon, it remains an amino acid. Its naming is therefore described in 3AA-2. This section extends some of the procedures described there, and also covers modification of functional groups.

A number of special procedures are given below to allow names to be based on the trivial names of the α-amino acids, so that they may indicate biochemical relationships. These procedures, which yield names such as N^6-lysino (3AA-7), alanin-3-yl (3AA-8), leucinamide (3AA-9.2), phenylalanylchloromethane (3AA-10.2), alaninol (3AA-10.3), etc., should not be extended to other areas.

3AA-6. IONIZATION OF FUNCTIONAL GROUPS AND NAMING OF SALTS

The predominant form at pH 7 of a mono-amino-mono-carboxylic acid is R–CH(NH_3^+)–COO^- rather than R–CH(NH_2)–COOH. It is nevertheless often convenient to draw the latter conventional form (e.g. in Table 1) and to name alanine as 2-aminopropanoic acid rather than as 2-ammoniopropanoate. This is particularly so for representing the isoelectric form of amino acids that contain other ionizing groups. A solution of lysine, for example, would contain appreciable amounts of both NH_3^+–$[CH_2]_4$–CH(NH_2)–COO and NH_2–$[CH_2]_4$–CH(NH_3^+)–COO^-.

When it is desirable to mention or stress the ionic nature of an amino acid, the three kinds of ions possible for a mono-amino-mono-carboxylic compound may be indicated as follows:

NH_3^+–CH_2–COO^-	glycine zwitterion (or dipolar ion, or amphion);
NH_3^+–CH_2–COOH	glycinium, or glycine cation;
NH_2–CH_2–COO^-	glycinate, or glycine anion.

In indicating an anion the ending 'ate' replaces 'ic acid' or the final 'e' of the trivial name, or is added to the name tryptophan.

Further forms are required for amino acids that contain ionizing side chains. The singly charged anions of aspartic and glutamic acids (strictly each has two negative and one positive charge, but this nomenclature refers to net charge) may be distinguished from the doubly charged anions by placing the charge after the name, or by stating the number of neutralizing ions.

Thus the form of glutamate (glutamate refers to glutamic acid; glutaminate is the anion from glutamine) with a charge of minus one, $^-OOC–CH_2–CH_2–CH(NH_3^+)–COO^-$, may be called glutamate(1-), glutamic acid monoanion, or hydrogen glutamate, and its sodium salt may be called sodium glutamate(1-), sodium hydrogen glutamate, or monosodium glutamate. The corresponding terms for the dianion, $^-OOC–CH_2–CH_2–CH(NH_2)–COO^-$, include glutamate(2-), glutamic-acid dianion, and disodium glutamate. Unqualified, the word glutamate systematically means the dianion; hence the usage 'sodium hydrogen glutamate'; in normal use, however, it means the ion of net charge -1, since this is the form that predominates in neutral solution, and it is used in this way in, for example, 'a glutamate-dependent reaction' and 'glutamate dehydrogenase'.

Similarly, forms such as lysinium(1+) or lysine monocation may be used for the ion of unit net charge derived from lysine. Its salts may be indicated by adding the name of the anion to the lysinium form, e.g. lysinium(1+) chloride, or by naming it lysine monohydrochloride. The fully protonated form is the lysine dication or lysinium(2+).

3AA-7. AMINO ACIDS SUBSTITUTED ON NITROGEN

Since N-2 is the atom most easily modified in many amino acids, the locant can often be omitted without ambiguity, e.g. acetylglycine for *N*-acetylglycine.

It is sometimes convenient to use the name of a group derived by loss of hydrogen from a nitrogen atom of an amino acid as a prefix in forming another name. Such prefixes are formed by substituting 'o' for the terminal 'e' in those names that end in 'e' (by analogy with amine→amino); e.g. alanino, valino. Tryptophan adds the 'o' directly, and the two dicarboxylic acids become asparto and glutamo. Where there is more than one nitrogen atom in the amino acid, a locant of the form N^x must precede the group name, e.g. N^{α}-lysino, N^{ω}-arginino, N^{δ}-glutamino, N^{π}-histidino.

3AA-8. SIDE-CHAIN MODIFICATIONS (excluding modifications of carboxyl or nitrogen)

Most modified amino acids can be named according to 3AA-2, e.g. *S*-(carboxymethyl)-L-cysteine. Groups formed by loss of hydrogen atoms from carbon, sulfur or oxygen atoms (excluding the carboxylic oxygen atoms, which are dealt with under 3AA-9) are named by substituting '-x-yl' for the terminal 'e' of the trivial name, where 'x' is the locant of the atom from which the hydrogen atom has been lost, e.g. cystein-*S*-yl, threonin-O^3-yl, alanin-3-yl, or by adding '-x-yl' to aspartic, glutamic and tryptophan, e.g. aspartic-2-yl, tryptophan-2-yl (see 3AA-2.2.3).

Comment. tryptophan-1-yl should be named 1-tryptophano according to 3AA-7.

A common side-chain modification is the oxidation of cysteine to yield cystine (formula in Appendix). Hydrogen atoms are removed from the –SH groups of two molecules, which are joined by an S–S bond. The term 'half cystine' refers to each half. It occurs seldom in naming compounds, since half a cystine molecule is a substituted cysteine and is named as such. In stating amount of substance, however, any specified entity may be used, so moles or numbers of residues of half cystine may usefully be compared with these quantities of other amino acids in stating protein composition.

3AA-9. ESTERS AND AMIDES OF THE CARBOXYL GROUP

3AA-9.1. *Esters*

Esters are named with the anion name (3AA-6), e.g. methyl prolinate, methyl cysteinate, or from the amino acid, e.g. proline methyl ester, cysteine methyl ester.

3AA-9.2. *Amides, Anilides and Analogous Derivatives* ($H_2N–CHR–CO–NH–R'$)

In amides, anilides and analogous derivatives of α-amino acids the hydroxyl group of the carboxyl has been replaced by an amino, anilino, or analogous group. They may be named by replacing the final 'e' of the trivial name of the amino acid by the word 'amide', 'anilide', etc., e.g. glycinamide, leucinamide, argininanilide. Alternatively, these compounds may be described as glycine amide, leucine amide, etc.

Note that the 4-amide of aspartic acid and the 5-amide of glutamic acid have specific trivial names, asparagine and glutamine. Their 1-amides are named aspartic 1-amide and glutamic 1-amide, or isoasparagine and isoglutamine.

3AA-9.3. *Acyl Groups*

The acyl group of an α-amino-mono-carboxylic acid is a structure that lacks the hydroxyl group of the carboxyl ($H_2N–CHR–CO–$). The names of such groups are formed by replacing the ending 'ine' (or 'an' in tryptophan) by 'yl' (C-421 of reference [14]), e.g. alanyl, arginyl, tryptophyl. 'Cysteinyl' is used instead of 'cysteyl', because of potential confusion with the group from cysteic acid. 'Cystyl' is the diacyl group of cystine, and 'half-cystyl' is the acyl group of cysteine lacking also the H of its SH group.

The monoacyl groups derived from aspartic acid, $HOOC–CH_2–CH(NH_2)–CO–$ and $–CO–CH_2–CH(NH_2)–COOH$, are designated α-aspartyl (or aspart-1-yl) and β-aspartyl (or aspart-4-yl) respectively; the corresponding groups derived from glutamic acid are α-glutamyl (or glutam-1-yl) and γ-glutamyl (or glutam-5-yl) (C-421.3 of reference [14]). The diacyl groups formed from the dicarboxylic amino acids are aspartoyl and glutamoyl. The acyl groups derived from asparagine and glutamine are termed asparaginyl and glutaminyl respectively.

3AA-10. CARBOXYL GROUP MODIFICATIONS OTHER THAN ESTER AND AMIDE FORMATION

3AA-10.1. *Removal of the Carboxyl Group*

Several decarboxylated amino acids have trivial names terminated with 'amine': tyramine, histamine, cysteamine, tryptamine, methioninamine. Similarly cystine (see Appendix) forms cystamine.

3AA-10.2. *Ketones*

▲ If the hydroxyl group of the 1-carboxyl is replaced by an alkyl group, the name of the ketone formed can use the name of the amino acid by naming the compound as a substituted hydrocarbon, e.g. phenylalanylchloromethane for $C_6H_5–CH_2–CH(NH_2)–CO–CH_2Cl$, 3-amino-1-chloro-4-phenylbutan-2-one (see also 3AA-18.2). This type of name is based on the trivial names of amino acids (or peptides), so does not place the substituents of methane in alphabetical order (as systematic nomenclature does), but places 'chloromethane' at the end because this indicates C-terminal modification (see 3AA-13.1). The practice of using names such as 'phenylalanine chloromethyl ketone' is discouraged, because they erroneously specify the carbonyl group twice.

3AA-10.3. *Aldehydes and Alcohols*

Aldehydes and alcohols obtained by successive stages of reduction of the carboxyl group of α-amino acids are named by replacing the final 'e' of a trivial name ending in 'ine' (or the 'ic acid' of aspartic and glutamic acids) with the endings 'al' and 'ol' respectively.

Examples. R–CH(NH_2)–CHO: alaninal, leucinal, lysinal, serinal, aspart-1-al, glutaminal. R–CH(NH_2)–CH_2OH: alaninol, leucinol, lysinol, serinol, aspart-1-ol, glutaminol. The aldehyde and alcohol derivatives of tryptophan take the names tryptophanal and tryptophanol. The name glycinol is little used, because the systematic name 2-aminoethanol is short, and this already has the trivial name ethanolamine [19].

Note. The derivative of lysine in which the $-CH_2-NH_2$ group is replaced by –CHO has the trivial name allysine (see Appendix).

Part 1, Section C: PEPTIDE NOMENCLATURE

3AA-11. DEFINITION OF PEPTIDES

A peptide is any compound produced by amide formation between a carboxyl group of one amino acid and an amino group of another. The amide bonds in peptides may be called peptide bonds. The word peptide usually applies to compounds whose amide bonds are formed between C-1 of one amino acid and N-2 of another (sometimes called eupeptide bonds), but it includes compounds with residues linked by other amide bonds (sometimes called isopeptide bonds). Peptides with fewer than about 10–20 residues may also be called oligopeptides; those with more, polypeptides. Polypeptides of specific sequence of more than about 50 residues are usually known as proteins, but authors differ greatly on where they start using this term.

3AA-12. AMINO-ACID RESIDUES

3AA-12.1. *Definitions of Residues*

When two or more amino acids combine to form a peptide, the elements of water are removed, and what remains of each amino acid is called an amino-acid residue. α-Amino-acid residues are therefore structures that lack a hydrogen atom of the amino group (–NH–CHR–COOH), or the hydroxyl moiety of the carboxyl group (NH_2–CHR–CO–), or both (–NH–CHR–CO–); all units of a peptide chain are therefore amino-acid residues. (Residues of amino acids that contain two amino groups or two carboxyl groups may be joined by isopeptide bonds, and so may not have the formulas shown.)

The residue in a peptide that has an amino group that is free, or at least not acylated by another amino-acid residue (it may, for example, be acetylated or formylated), is called N-terminal; it is at the N-terminus. The residue that has a free carboxyl group, or at least does not acylate another amino-acid residue, (it may, for example, acylate ammonia to give –NH–CHR–CO–NH_2), is called C-terminal. If the amino group of the N-terminal residue is free, the residue may be named as an acyl group under 3AA-9.3; indeed any internal residue is an *N*-substituted amino-acyl group.

Residues are named from the trivial name of the amino acid, omitting the word 'acid' from aspartic acid and glutamic acid. Examples: glycine residue, lysine residue, glutamic residue.

3AA-12.2. *Ionized Forms of Residues*

When it is desirable to mention or emphasize the particular ionic form of a residue, this may be done as follows

Name of residue	*Protonated form*	*Deprotonated form*
arginine residue	argininium residue	arginine (base) residue
histidine residue	histidinium residue	histidine (base) residue
lysine residue	lysinium residue	lysine (base) residue
aspartic residue	aspartic (acid) residue	aspartate residue
cysteine residue	cysteine (acid) residue	cysteinate residue
glutamic residue	glutamic (acid) residue	glutamate residue
tyrosine residue	tyrosine (acid) residue	tyrosinate residue

This system cannot easily be applied to N- or C-terminal residues.

3AA-13. THE NAMING OF PEPTIDES

3AA-13.1. *Construction of Names*

To name peptides, the names of acyl groups ending in 'yl' (3AA-9.3) are used. Thus if the amino acids glycine, NH_3^+–CH_2–COO^-, and alanine, NH_3^+–$CH(CH_3)$–COO^-, condense so that glycine acylates alanine, the dipeptide formed, NH_3^+–CH_2–CO–NH–$CH(CH_3)$–COO^-, is named glycylalanine. If they condense in the reverse order, the product, NH_3^+–$CH(CH_3)$–CO–NH–CH_2–COO^-, is named alanylglycine. Higher peptides are named similarly, e.g. alanylleucyltryptophan. Thus the name of the peptide begins with the name of the acyl group representing the N-terminal residue, and this is followed in order by the names of the acyl groups representing the internal residues. Only the C-terminal residue is represented by the name of the amino acid, and this ends the name of the peptide. Formulas should normally be written in the same order, with the N-terminal residue on the left, and the C-terminal on the right, e.g.

$$NH_2\text{–}CH(COOH)\text{–}[CH_2]_2\text{–}CO\text{–}NH\text{–}CH(CH_2SH)\text{–}CO\text{–}NH\text{–}CH_2\text{–}COOH$$

glutathione, γ-glutamylcysteinylglycine

A multiplicative affix (p. 5 of reference [14]) placed before 'peptide' gives the total number of residues in the peptide, e.g. ▲ hexapeptide. Since the higher affixes are not well known, they may be replaced by numerals, e.g. a 22-peptide.

Higher oligopeptides and polypeptides of biological origin often have trivial names; their sequences are usually described more conveniently by symbols (3AA-14 to 3AA-19 below) than by constructing long names.

3AA-13.2. *Use of Prefixes in Peptide Names*

Configurational prefixes (3AA-3) are placed immediately before the trivial names of the residues they refer to. The prefixes are set off from the names before and after them with hyphens. Examples: L-alanyl-L-leucine; L-alanyl-D-leucine; glycyl-L-alanine; L-alanylglycine; L-leucyl-L-phenylalanyl-L-leucylglycine; L-alanylglycyl-L-leucine.

▲ The mixture of diastereoisomers formed by condensations between DL-amino acids will contain unspecified proportions of each pair of enantiomers. Names such as DL-alanyl-DL-leucine have been used in the past, but they are misleading because they

contradict the accepted meaning of the prefix DL as signifying a racemate; here the racemate of L-alanyl-L-leucine and D-alanyl-D-leucine (which may be designated as *rac*-L-alanyl-L-leucine) is mixed in unspecified proportions with the racemate of L-alanyl-D-leucine and D-alanyl-L-leucine (which may similarly be designated as *rac*-L-alanyl-D-leucine). This is better indicated by the name *ambo*-alanyl-*ambo*-leucine (item 12c of reference [20]).

A mixture of L-alanyl-L-alanyl-L-alanine and L-alanyl-D-alanyl-L-alanine may likewise be called L-alanyl-*ambo*-alanyl-L-alanine.

3AA-13.3. *Name of Simple Polymers of α-Amino Acids*

Simple polymers of amino acids may, if preferred, be named with prefixes to indicate the number of amino-acid residues present, e.g. tetraglycine. Mixtures of polymers with varying numbers of residues may be given names like oligoglycine, polyglycine, poly(L-lysine), etc. [21].

3AA-13.4. *Numbering of Peptide Atoms*

The atoms of a peptide may need to be numbered as locants for substitution or isotopic replacement. Often no more numbering is required than that of atoms within a residue (see 3AA-2.2), e.g. alanyl-3-chloroalanylalanine. It may sometimes be convenient to indicate substitution of the peptide as a whole. This may be done by adding the residue number, obtained by ▲ numbering residues from the N-terminus, after the atom number, and separated from it by a point. The above compound may therefore be called 3.2-chloro(alanylalanylalanine). Thus the atom C-3.2 is C-3 of the second residue of the peptide. Example: Alanylthreonylglycylaspartylglycine 4.4-3.2-lactone for the compound that can be represented (3AA-16, -17 and -19 below) as Ala-Thr-Gly-Asp-Gly.

Such numbering is especially useful for peptides with trivial names (see 3AA-22.5), e.g. $N^{5,4}$-methyloxytocin would indicate a methyl substituent on N-5 of the glutamine residue at position 4 of oxytocin. If the peptide name that follows a substituent indicated in this way is constructed residue by residue, it must be placed in parentheses to show that the numbering applies to the peptide as a whole, rather than to the first residue.

3AA-13.5. *Prefixes Formed from Peptide Names*

▲ When it is necessary to treat a peptide as a substituent, the point of attachment is specified by the suffix 'yl' (see 3AA-8) with the appropriate locant. If the group formed from the peptide is not the acyl group derived by removing hydroxyl from C-1 of the C-terminal residue, the position at which hydrogen (or hydroxyl from a side-chain carboxyl group) is removed should be indicated by a locant before the 'yl'; if the sequence of the peptide is given in full, it should be placed in parentheses to avoid implying that the group is formed by removing H or OH from the C-terminal residue. Examples:

(1) Leukotriene D, or (7*E*,9*E*,11*Z*,14*Z*)-(5*S*,6*R*)-6-[(cysteinylglycin)-*S*-yl]-hydroxyicosa-7,9,11,14-tetraenoic acid;

(2) Leukotriene C, or (7*E*,9*E*,11*Z*,14*Z*)-(5*S*,6*R*)-6-(glutathion-*S*-yl)-5-hydroxyicosa-7,9,11,14-tetraenoic acid, or (7*E*,9*E*,11*Z*,14*Z*)-(5*S*,6*R*)-6-[(γ-glutamylcysteinylglycin)-*S*-yl]-5-hydroxyicosa-7,9,11,14-tetraenoic acid;

(3) (2*S*)-2-*O*-[(serylalanylserin)-3,2-yl]lactic acid, or (2*S*)-2-[(serylserylserin)-$O^{3,2}$-yl]propanoic acid, or $O^{3,2}$-[(1*S*)-1-carboxyethyl](serylserylserine).

If the locant before 'yl' indicates the carbon of a carboxyl group, the prefix indicates the acyl group formed by removing hydroxyl from this atom. Example: 4-*O*-[(glutamylglutamylglutamic acid)-5,2-yl]-D-gluconic acid.

3AA-13.6. *Conformation of Polypeptide Chains*

Abbreviations and symbols for describing the conformation of peptide chains have been published separately [16].

Part 2. **Symbolism**

Part 2, Section A: THE THREE-LETTER SYSTEM (a revision and updating of [10])

3AA-14. GENERAL CONSIDERATIONS ON THREE-LETTER SYMBOLS

14.1. The symbol chosen for an amino acid (Table 1) is derived from its trivial name, and is usually the first three letters of this name. It is written as one capital letter followed by two lower-case letters, e.g. Gln (not GLN or gln), regardless of its position in a sentence or structure. If any other convention is used in representing residues, e.g. to emphasize homology, this should be stated clearly whenever it is used. When the symbol is used for a purpose other than representing an amino-acid residue, e.g. to designate a genetic factor, three lower-case italic letters may be used, e.g. *gln*.

14.2. The main use of the symbols is in representing amino-acid sequences. Inasmuch as the symbols by themselves represent the unsubstituted amino acids, they are modified (3AA-16) by hyphens to represent residues. We do not recommend use of the symbols to represent free amino acids in textual material, but such use may be desirable in tables, diagrams or figures. It may also be convenient to use them for indicating residue numbers, e.g. Tyr-110 for tyrosine residue 110. For substituents, supplementary symbols are used (3AA-17 and -18).

14.3. A symbol may represent either the name or the formula of a compound.

14.4. Heteroatoms of amino-acid residues (e.g. O-3 serine, N-6 of lysine) do not explicitly appear in the symbol, as it represents the whole molecule including them (but see 3AA-17.4).

14.5. Amino-acid symbols denote the L configuration of chiral amino acids unless otherwise indicated by the presence of D or DL before the symbol and separated from it with a hyphen (see also 3AA-19.2). L may similarly be inserted for emphasis.

14.6. Structural formulas may be used together with symbols to make complicated features or reactions clear (for examples see 3AA-17.4).

3AA-15. SYMBOLS FOR AMINO ACIDS

3AA-15.1. *Symbols for Common Amino Acids*

The symbols for the amino acids that are coded for by mRNA are listed in column 2 of Table 1.

3AA-15.2. *Symbols for Less Common Peptide Constituents*

Symbols for less common amino acids should be defined in each publication in which they appear. The following principles and notations are recommended.

15.2.1. Hydroxyamino Acids

The symbol 5Hyl is recommended for 5-hydroxylysine, and 4Hyp for 4-hydroxyproline (the numbers may be omitted, especially when limiting the symbols to three letters helps alignment of sequences, provided that the position of substitution is made clear in the text). Similarly 3Hyp would represent 3-hydroxyproline. Alternatively, symbols may be formed as shown in 3AA-17.3 below for substituted residues, so that 4-hydroxyproline may be written as:

Pro(4-OH) or $\overset{\text{OH}}{\underset{4}{\text{Pro}}}$

15.2.2. Alloisoleucine and Allothreonine

Alloisoleucine and allothreonine (3AA-4.4) may be symbolized by alle and aThr respectively.

15.2.3. 'Nor' Amino Acids

Since 'nor' in 'norvaline' and 'norleucine' is not used in its systematic sense of denoting a lower homologue, but to change the trivial name of a branched-chain compound to designate a straight-chain compound, its use for amino acids should be ▲ *progressively* abandoned (3AA-2.4), along with the earlier symbols Nva and Nle. Appropriate symbols for these compounds, 2-aminovaleric and 2-aminohexanoic acids, based on symbols proposed for the unsubstituted acids [19], are Avl and Ahx (see also 3AA-15.2.5).

15.2.4. 'Homo' Amino Acids

The prefix 'homo', used in the sense of a higher homologue, is commonly used for two amino acids (3AA-2.3). They are symbolized as follows:

Homoserine	Hse
Homocysteine	Hcy

15.2.5. Higher Unbranched Amino Acids

The functional prefix 'amino' is included in the symbol as the letter 'A' and 'diamino' as 'A_2'. The trivial name of the parent acid is abbreviated to two letters, based, when possible, on the symbols for lipid nomenclature [19]. Unless otherwise indicated, single groups are on C-2, two amino groups are in the 2 and terminal positions for monocarboxylic acids, and each is geminal with a carboxyl group for dicarboxylic acids. The location of amino groups other than these is shown by appropriate prefixes.

	Examples	*Symbol*	*Note*
	β-Alanine (3-aminopropanoic acid)	βAla	
	2-Aminobutyric acid (2-aminobutanoic acid)	Abu	
▲	2-Aminovaleric acid (2-aminopentanoic acid)	Ape	
	2-Aminohexanoic acid	Ahx	
▲	6-Aminohexanoic acid	εAhx	i
	2-Aminoadipic acid (2-aminohexanedioic acid)	Aad	
	3-Aminoadipic acid (3-aminohexanedioic acid)	βAad	
	2-Aminopimelic acid (2-aminoheptanedioic acid)	Apm	
▲	2,3-Diaminopropionic acid (2,3-diaminopropanoic acid)	A_2pr or Dpr	ii, iii, iv
▲	2,4-Diaminobutyric acid (2,4-diaminobutanoic acid)	A_2bu or Dab	ii
	Ornithine (2,5-diaminovaleric acid, 2,5-diaminopentanoic acid)	Orn	
▲	2,6-Diaminopimelic acid (2,6-diaminoheptanedioic acid)	A_2pm or Dpm	ii, iii

Notes

i) This symbol is recommended in place of the previous εAcp, in which 'cp' stood for caproic, which may be confused with capric and caprylic.

ii) The previous edition of these recommendations (10) discouraged abbreviations starting 'D' for 'di' or 'T' for 'tri' or 'tetra'; because these letters were overused. We concur in preferring subscripts when these can be applied to well-known symbols, so that Me_2SO is preferable to DMSO, Me_3Si- to TMS-, and H_4 to TH. Nevertheless we are not convinced that 'A_2' easily suggests 'diamino', so alternative symbols are presented.

iii) 'Dap' should not be used as a symbol, since it could be construed to mean either diaminopropanoic acid or diaminopimelic acid.

iv) 2,3-Diaminopropanoic acid can be regarded as 3-aminoalanine, and so may be symbolized by 'side-chain substitution' (3AA-17.3 below) as Ala(NH_2) or Ala, but users should beware of the possibility that the former may be confused with Ala-$\overset{|}{\underset{NH_2}{}}$ (3AA-17.1), the symbol for alaninamide.

15.2.6. Carboxylated and Oxidized Amino Acids

Symbols are recommended for two amino acids that have an additional acidic group and may occur in polypeptide sequences. They are:

4-Carboxyglutamic acid	Gla
Cysteic acid	Cya

15.2.7. Non-Amino-Acid Residues in Peptides

Symbols for sugar residues (e.g. Glc, Gal) have been proposed [22], as have ones for nucleoside residues (e.g. Ado, Cyd) [23], and these may be combined with amino-acid symbols to represent glycopeptides, etc. These symbols include [22] Neu for

neuraminic acid, Neu5Ac for *N*-acetyl neuraminic acid, and Mur for muramic acid. Depsipeptides (3AA-19.6) contain hydroxy-acid residues; when symbols are used for these they should be defined.

3AA-16. SYMBOLISM OF AMINO-ACID RESIDUES

3AA-16.1. *General Principles for Symbolizing Residues*

The peptide glycylglycylglycine is symbolized as Gly-Gly-Gly. This involves modifying the symbol Gly for glycine, NH_2–CH_2–COOH, by adding hyphens to it, in three ways:

i) Gly-= NH_2–CH_2–CO– (normally as NH_3^+–CH_2–CO–)

ii) -Gly = -NH–CH_2–COOH (normally as –NH–CH_2–COO^-)

iii) -Gly-= -NH–CH_2–CO–

Thus the hyphen, which represents the peptide bond, removes OH from the 1-carboxyl group of the amino acid (written in the conventional un-ionized form) when it is placed on the right of the symbol (i), and removes H from the 2-amino group of the amino acid when it is placed on the left of the symbol (ii); both modifications can apply to one symbol (iii).

Thus the peptide Gly-Glu (without hyphens at its ends) is distinguished from the sequence -Gly-Glu- (with hyphens at its ends).

3AA-16.2. *Lack of Hydrogen on the 2-Amino Group*

A hyphen on the left of the symbol signifies removal of a hydrogen atom from the 2-amino group, as well as representing the bond formed by the group thus produced. If it should prove necessary to draw a bond to N-2 on the right of the symbol (e.g. in a cyclic peptide, 3AA-19.4 below), then the hyphen must be replaced by an arrow, which points from CO to NH within the peptide bond.

If both atoms on N-2 are replaced, two lines can be drawn on the left of the symbol, e.g.

$\gtrdot$Ala or $\perp$Ala for $\gtrdot$N–CH(CH_3)–COOH

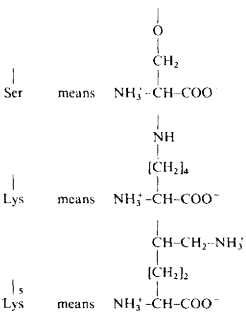
3AA-16.3. *Lack of Hydroxyl on the 1-Carboxyl Group*

A hyphen on the right of the symbol signifies removal of hydroxyl from the 1-carboxyl group as well as representing the bond formed by the group produced. If it is not possible to draw this bond on the right of the symbol, as in a cyclic peptide (3AA-19.4) then the hyphen must be replaced by an arrow, which has the same effect.

3AA-16.4. *Removal of Groups from Side Chains*

16.4.1. Monocarboxylic Acids

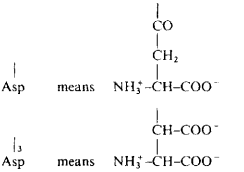
A vertical line drawn above or below the symbol for a monocarboxylic amino acid represents removal of hydrogen from the side chain so that a radical is formed. Replacement of this hydrogen by a substituent is treated in 3AA-17.2 below. Unless indicated by a locant placed beside the line, the hydrogen is assumed to be removed from a heteroatom in the residue. Examples:



Notes. (a) H is removed from N-ω rather than N-δ of arginine unless otherwise indicated; (b) a locant, π or τ (3AA-2.2.4), is always required for histidine.

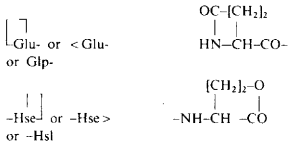
16.4.2. Dicarboxylic acids

A vertical line drawn above or below either of the symbols Asp and Glu represents removal of OH from the side-chain carboxyl group, as well as representing a bond to a substituent. If a hydrogen has to be removed from a saturated carbon of the side chain, then a vertical line may be used, but it must be accompanied by a locant. Examples:



3AA-16.5. *Cyclic Derivatives of Amino Acids*

Combination of horizontal lines, indicating removal of H from N-2 (3AA-16.1, 3AA-16.2) or OH from C-1 (3AA-16.1, 3AA-16.3), with the vertical lines that indicate removal of side-chain atoms (3AA-16.4) allows formation of symbols for 5-oxoproline (systematically 5-oxopyrrolidine-2-carboxylic acid, also known as pyroglutamic acid or pyrrolidonecarboxylic acid) and for homoserine lactone, as follows:



3AA-17. SUBSTITUTED AMINO ACIDS

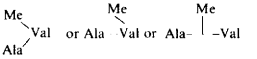
3AA-17.1. *Substitutions in the 2-Amino and 1-Carboxyl Groups*

This follows logically from 3AA-16.1, 3AA-16.2 and 3AA-16.3 by using symbols for atoms or groups to represent the substituents. Examples (see also 3AA-18.2):

N-Acetylglycine	Ac-Gly
Glycine ethyl ester	Gly-OEt
N^2-Acetyllysine	Ac-Lys
Serine methyl ester	Ser-OMe
O^1-Ethyl *N*-acetylglutamate	Ac-Glu-OEt
Isoglutamine	Glu-NH_2
O^1-Methyl hydrogen aspartate	Asp-OMe

A second substituent on N-2, when the first is shown with a line as above, may be represented with a second line to the left of the symbol for the substituted residue: >Xaa. It may be convenient to print this as a vertical line joining the first: ⊥Xaa.

Example: alanyl-*N*-methylvaline may be represented



3AA-17.2. *Substitutions on Side-Chain Functional Groups*

Side-chain substituents may be portrayed above or below the amino-acid symbol (3AA-16.4) or by placing the symbol for the substituent in parentheses immediately after the amino-acid symbol. When the symbol for a substituent, such as an oligosaccharide, has a hyphen on its right-hand side to indicate the bond to the amino acid [22], then this symbol should be placed in parentheses before the amino-acid symbol rather than after it, e.g. -(Galβ1-4Xylβ1-)Ser- [22].

Symbols within parentheses written on one line should normally be used only in textual material and when the symbol for the substituent is short; otherwise the two-line symbols containing a vertical line will be clearer. Note that the substituents represented replace hydrogen except when the amino acid is aspartic acid or glutamic acid, when they replace the OH of the carboxyl group unless otherwise specified (3AA-16.4.2).

If a locant is required, it is placed beside the vertical line that represents side-chain substitution, or is joined to the substituent symbol within the parentheses by a hyphen.

Examples	*Symbols*	*Notes*
O^4-Methyl hydrogen aspartate (β-methyl aspartate)	Asp(OMe)	i
O^5-Ethyl hydrogen *N*-acetylglutamate	Ac–Glu(OEt)	i
N^6-Acetyllysine	Lys(Ac)	
O^3-Acetylserine	Ser(Ac)	
O^4-Sulfotyrosine (tyrosine O^4-sulfate)	Tyr(SO_3H)	
S-Ethylcysteine	Cys(Et)	
3-Sulfenoalanine	Cys(OH)	ii
S-Sulfocysteine (*S*-cysteinesulfonic acid)	Cys(SO_3H)	ii, iii
S-Cyanocysteine	Cys(CN)	

Cystine	Cys or Cys Cys (not Cys-Cys)	
D-Cystine	D-Cys D-Cys	
meso-Cystine	L-Cys D-Cys or D-Cys L-Cys	
▲ Methionine *S*-oxide (methionine oxide)	Met(O) or MetO	ii, iv
▲ Methionine *S,S*-dioxide (methionine dioxide)	Met(O_2) or MetO_2	ii, iv
Phosphoserine (O^3-phosphonoserine)	Ser(*P*) or Ser(*P*)	v
N^{τ}-Methylhistidine (*tele*methylhistidine, see 3AA-2.2.4)	His(τ-Me) or His(τ-Me)	

Notes

i) Asp-OMe represents the O^1-methyl ester of aspartic acid (C-1 modification by 3AA-16.3), whereas Asp(OMe) represents the O^4-methyl ester (side-chain modification by 3AA-16.4.2).

ii) Names based on cysteine and symbols based on Cys already indicate sulfur in the molecule, and similarly with methionine and Met. Indication of modification of this sulfur should not suggest the addition of further sulfur. Hence calling 3-sulfenoalanine by the name cysteinesulfenic acid, 3-sulfinoalanine by the name cysteinesulfinic acid, methionine *S*-oxide by the name methionine sulfoxide, and methionine *S,S*-dioxide by the name methionine sulfone may be confusing and is not recommended.

iii) Care should be taken with this symbol because readers who fail to realize that the symbol Cys contains the sulfur may confuse it with cysteic acid, now symbolized Cya (3AA-15.2.6). The earlier [10] symbol Cys for cysteic acid has the disadvantage that the vertical line in it does not represent a single bond.

iv) The vertical lines or parentheses previously [10] in the symbols for MetO and MetO_2 are now omitted, because they wrongly implied removal of hydrogen.

v) –*P* represents $-PO_3H_2$ [24].

3AA-17.3. *Substitution on Side-Chain Skeleton*

This may use the same convention as 3AA-17.2, with the addition of locant numerals where necessary (see 3AA-16.4), e.g.

4-Carboxyglutamic acid	Glu as an alternative to Gla (3AA-15.2.6) if it is desired to emphasize carboxylation
2,3-Diaminopropanoic acid (3-aminoalanine, see 3AA-15.2.5)	Ala or Ala(NH_2)
3,5-Diiodotyrosine	Tyr(I_2) or Tyr(3,5-I_2) if the context does not imply the locants
3-Nitrotyrosine	Tyr

3AA-17.4. *The Use of Symbols in Representing Reactions of Side-Chains*

▲ The symbols are designed primarily to indicate sequence, and care must be taken to avoid confusion when they are adapted to other uses.

Although the conversion of a cysteine residue in a protein into an *S*-carboxymethylcysteine can be adequately represented as

$$\text{–Cys–} \longrightarrow \text{–Cys(CH}_2\text{–COOH)–}$$

writers may wish to show the sulfur atom in order to indicate the chemistry of the reaction. Although it would be perfectly legitimate to write

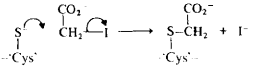
$$\text{–Ala(SH)–} \longrightarrow \text{–Ala(S–CH}_2\text{–COOH)–}$$

this would be confusing since the residue is thought of as cysteine rather than as modified alanine.

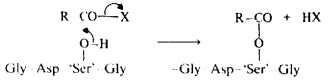
We therefore recommend putting the residue symbol into quotation marks if one of its groups is to be depicted separately (or, alternatively, using the symbol R_{Xaa}). Hence the thiol group of a cysteine residue may be shown as:

$$\text{–'Cys'–} \quad \text{or} \quad \text{–R}_{\text{Cys}}\text{–}$$

Terminal amino and carboxyl groups can be shown similarly, e.g. H_2N–'Ala'– to show explicitly the amino group present in Ala- (in contrast with –Ala–NH_2 which shows the amide of C-terminal alanine). This convention allows mechanisms to be drawn out, e.g.



with the quotation marks to alert readers to the fact that the symbol here does not include the sulfur atom. Sequences may also be shown, so that the acylation of a serine proteinase could be drawn as:



When this convention is used it should be described.

3AA-17.5. *Modified Residue in Natural Peptides*

▲ If an unusual residue is to be symbolized within a particular context, it may be helpful to modify (e.g. with an asterisk) the symbol for the ribosomally incorporated residue, e.g. Ser* for 2-aminopropenoic acid (formed within a peptide chain by dehydration of a serine residue). Such an asterisk may be placed above the residue rather than after it to allow alignment with other 3-letter symbols. Symbols modified in this way should be defined when used.

3AA-17.6. *Lack of Substitution*

If it is desired to emphasize lack of substitution, H or OH may be added to the hyphen or vertical line that represents removal of one of these groups. Thus H-Ala may be contrasted with Ac-Ala, and Ala-OH with Ala-OMe.

3AA-18. SYMBOLS FOR SUBSTITUENTS

3AA-18.1. *Use of Symbols*

Groups substituted for hydrogen or hydroxyl may be indicated by their formulas or by symbols or by combination of both, e.g.

Benzoylglycine (hippuric acid) PhCO-Gly or C_6H_5CO-Gly

Note: the symbol Bz is often used for benzoyl in organic chemistry, and Bzl for benzyl, but because these symbols are so similar, the alternative PhCO and $PhCH_2$ are preferable.

Glycine methyl ester Gly–OCH_3 or Gly–OMe
Trifluoroacetylglycine CF_3CO–Gly (Table 3, Note ii)

Suggestions for symbols to designate substituent (or protecting) groups common in peptide and protein chemistry are given in Tables 2, 3 & 4.

Table 2. *Nitrogen substituents (protecting groups) of the urethane type*

Benzyloxycarbonyl-	Z- or Cbz-
2-(*p*-Biphenylyl)isopropyloxycarbonyl- [strictly 1-(biphenyl-4-yl)-1-methylethoxycarbonyl-]	Bpoc-
p-Bromobenzyloxycarbonyl-	Z(Br)-
t-Butoxycarbonyl-	Boc- or Bu'OCO- or t-BuOCO- or Me_3C–OCO-
α,α-Dimethyl-3,5-dimethoxybenzyloxycarbonyl-	Ddz-
Fluoren-9-ylmethoxycarbonyl-	Fmoc-
p-Methoxybenzyloxycarbonyl-	Z(OMe)-
p-Nitrobenzyloxycarbonyl-	$Z(NO_2)$-
p-Phenylazobenzyloxycarbonyl-	Pz-

Table 3. *Non-urethane substituents for nitrogen, oxygen or sulfur*

Acetamidomethyl-	Acm-
Acetyl	Ac-
Benzoyl- (C_6H_5–CO-)	PhCO- (or Bz-; see note in 3AA-18.1)
Benzyl- (C_6H_5–CH_2-)	$PhCH_2$- (or Bzl; see note in 3AA-18.1)
Carbamoyl-	NH_2CO- (preferred to Cbm-)
(3-Carboxy-4-nitrophenyl)thio-	Nbs- (see 3AA-18.2)
3-Carboxypropanoyl- (HOOC–CH_2–CH_2–CO-)	Suc- (see Note i)
Dansyl-, 5-(dimethylamino)naphth-1-ylsulfonyl-	Dns-
2,4-Dinitrophenyl-	Dnp- or N_2ph (see Note ii)
Formyl-	HCO- or For- (see Note iii)
4-Iodophenylsulfonyl- (pipsyl-)	Ips-
Maleoyl- (-OC–CH=CH–CO-)	-Mal- or Mal< (C-404.1 of [14])
Maleyl- (HOOC–CH=CH–CO-)	Mal-
2-Nitrophenylthio-	NpS- (Nps- often used)
Phenyl(thiocarbamoyl)-	PhNHCS- or Ptc-
Phthaloyl-	-Pht- or Pht<
Phthalyl- (*o*-carboxybenzoyl-)	Pht-
Succinyl- (-OC–CH_2–CH_2–CO-)	-Suc- or Suc< (see Note i)
Tosyl-	Tos-
Trifluoroacetyl-	CF_3CO-
Trityl- (triphenylmethyl-)	Ph_3C- or Trt-

Notes

i) In organic nomenclature (C-404.1 of [14]), 'succinyl' signifies the bivalent group formed from succinic acid by removal of both hydroxyl groups, but in biochemical usage it usually signifies the 3-carboxypropanoyl group, e.g. succinyl-CoA.

ii) The use of D for 'di' and T for 'tri' and 'tetra' is discouraged if these apply to atoms or groups for which simple symbols exist, e.g. in CF_3CO–, Me_3Si– and H_4folate. We feel less strongly when their avoidance involves giving unusual meanings to symbols, e.g. N for nitro, so Dnp and N_2ph are offered as alternative symbols for dinitrophenyl. See also Note ii of 3AA-15.2.5.

iii) The symbol HCO– is preferred to CHO– for the formyl group, because CHO– has sometimes been used to indicate the attachment of carbohydrate.

3AA-18.2. *Principles of Symbolizing Substituent Groups and Reagents*

Many reagents used in peptide and protein chemistry for modifying (often protecting) amino, carboxyl and side-chain groups in amino-acid residues have been designated by a variety of acronymic abbreviations, too numerous to list here. Extensive and

▲ Table 4. *Substituents at the carboxyl group*

Group	Symbol	Name of glycine derivative (see note)
Benzotriazol-1-yloxy	–OBt	1-(Glycyloxy)benzotriazole
Benzyloxy	$-OCH_2Ph$ (or–OBzl, see note in 3AA-18.1)	Glycine benzyl ester
tert-Butoxy	$-OCMe_3$ or –OBu^t	Glycine *t*-butyl ester
Diphenylmethoxy	$-OCHPh_2$ or –OBzh	Glycine diphenylmethyl ester (or benzhydryl ester)
Ethoxy	–OEt	Glycine ethyl ester
Methoxy	–OMe	Glycine methyl ester
4-Nitrobenzyloxy	–ONb	Glycine 4-nitrobenzyl ester
4-Nitrophenoxy	–ONp	Glycine 4-nitrophenyl ester
4-Nitrophenylthio	–SNp	Thioglycine *S*-(4-nitrophenyl ester)
Pentachlorophenoxy	–OPcp	Glycine pentachlorophenyl ester
Phenylthio	–SPh	Thioglycine *S*-(phenyl ester)
Quinolin-8-yloxy	–OQu	Glycine quinolin-8-yl ester
Succinimido-oxy	–ONSu or –OSu	*N*-(Glycyloxy)succinimide
2,4,5-Trichlorophenyloxy	–OTcp	Glycine 2,4,5-trichlorophenyl ester

Note. Carboxyl substituents will not normally appear as prefixes in the names of derivatives of amino acids or peptides, so the name of the group, its prefix name, given in column 1, is little used in naming compounds. Column 3 is therefore given to show how derivatives containing the group are named (by one of the alternative methods of 3AA-9.1).

indiscriminate use of such abbreviations is discouraged, especially when the accepted trivial name of the reagent is short, e.g. tosyl chloride, trityl chloride, etc.

It can be useful to symbolize a reagent in such a way that the group transferred retains its identity in a reaction, e.g.

$$\text{Dns–Cl} + \text{Gly} \rightarrow \text{Dns–Gly} + H^+ + Cl^-$$
$$\text{Dnp–F} + NH_2\text{–R} \rightarrow \text{Dnp–NH–R} + H^+ + F^-$$

For this reason Dns–Cl is usually preferred to DNS for dansyl chloride (although the full name is short enough for most textual use), and Dnp–F to the original FDNB for 1-fluoro-2,4-dinitrobenzene, and similarly Nbs_2 in place of DTNB for 3,3′-dithiobis(6-nitrobenzoic acid) (Ellman's reagent) and $(Pr^iO)_2PO$–F or Dip–F for diisopropyl fluorophosphate.

Symbols constructed from known elements are more readily understood than arbitrary abbreviations, e.g. Tos-Arg-OMe rather than TAME for tosylarginine methyl ester, and Tos-Phe-CH_2Cl rather than TPCK for 'tosylphenylalanine chloromethyl ketone', a name incorrectly used for tosylphenylalanylchloromethane (3AA-10.2), but misleading because it erroneously specifies the carbonyl group twice.

3AA-19. PEPTIDE SYMBOLISM

3AA-19.1. *Peptide Chains*

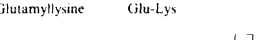
The amino-acid symbols were developed for representing peptide sequences (3AA-16). Peptides containing bonds other than between C-1 and N-2 of adjacent residues are also easily represented (3AA-16 to -18). Examples:

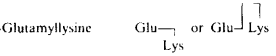
Glycylglycine	Gly-Gly
N-α-Glutamylglycine	Glu-Gly
N-γ-Glutamylglycine	Glu(Gly) or Glu(Gly) or Glu(Gly) or Glu(-Gly)
Thyroliberin	Glp-His-Pro-NH_2
Angiotensin II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
Glutathione	Glu(Cys-Gly) or Glu(Cys-Gly) or Glu(-Cys-Gly)

Note. Glu(Cys-Gly) would represent the corresponding thiol ester with a bond between the γ-carboxyl of glutamic acid and the thiol group of cysteine.

N^2-α-Glutamyllysine Glu-Lys

N^6-α-Glutamyllysine

N^2-γ-Glutamyllysine 

N^6-γ-Glutamyllysine 

Symbols for modified residues or names of compounds may be used in such formulas. Thus a peptide with a C-terminal aldehyde may be shown using either a name or a symbol constructed according to 3AA-16.3. Example:

▲ Ac-Leu-Leu-argininal or Ac-Leu-Leu-Arg-H

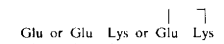
(If the second method is used, the symbol should be explained to avoid confusion.)

▲ If part of a sequence is unknown, but its composition can be specified, this may be indicated by parentheses, with commas between the residues listed as present, e.g. Ala-Lys-(Ala,Gly_3,Val_2)-Glu-Val.

▲ If a peptide must be written on more than one line, we advise placing a hyphen at the end of each line to be continued (where it has its usual meaning of a continuation symbol), and also at the start of the next line (where it represents the peptide bond), e.g.

Ala-Ser-Tyr-Phe-Ser-
-Gly-Pro-Gly-Trp-Arg

In diagrams the two lines can usually be joined, as in



but such a break may also be needed in textual material where this is not possible.

3AA-19.2. *Use of Configurational Prefixes*

Residue symbols written in a sequence denote the L configuration for chiral amino acids, unless otherwise indicated (3AA-14.5). A D residue is shown by inserting a D before the symbol, separated from it by a hyphen (which may be omitted to make the number of residues appear more clearly).

The symbol DL signifies a racemic mixture, so should not occur in the designation of peptides with more than one chiral residue; coupling of a DL-amino acid with a chiral peptide leads to a mixture of diastereoisomeric products whose ratio may ▲ depend on the conditions of the reaction and will not in general be unity. To indicate that both are present, *ambo* may be used (3AA-13.2), and thus the mixture of products formed by acylating L-leucine with DL-alanine may be represented as *ambo*-Ala-Leu, and a mixture of Phe-Ala-Leu and Phe-D-Ala-Leu may be represented as Phe-*ambo*-Ala-Leu.

▲ A residue of unknown configuration may be indicated by the prefix ξ (Greek xi), e.g. ξ-Ala.

3AA-19.3. *Representation of Charges on Peptides*

▲ It is usually convenient to use the same abbreviated formula for a peptide regardless of its state of ionization. To indicate or stress the charges on a peptide, plus and minus signs may be placed over residues with charged side chains and on either side of the formula to represent charged termini, e.g.

$^+$Gly-Glu-Ala-Lys-Cys-Val$^-$ and ist form in alkali: Gly-Glu-Ala-Lys-Cys-Val$^-$

Such signs may be circled for clarity.

If, however, it is desired to indicate charge by formal modification of the symbols for residues, this may be done as follows.

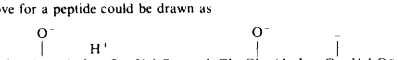
i) *Protonation of the N-terminus.* The sign $^+$H is placed beside the symbol for the N-terminal residue without a hyphen between (since a hyphen would signify removal of H). This gives, for example, $^+$HGly-. We prefer this to the alternative recommendation [10] of adding $^+H_2$-, to give, for example, $^+H_2$-Gly-, because it seems artificial to remove one hydrogen before adding two, and because the hyphen here fails to represent a single bond.

ii) *Deprotonation of the C-terminus.* The symbol -O^- is placed on the right of the C-terminal residue. Its hyphen signifies removal of -OH from the carboxyl group, so this is replaced by -O^-.

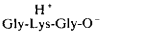
iii) *Protonation of Side-Chain basic groups.* 'H^+' is placed above the amino-acid symbol in the two-line representation, or after it, e.g. LysH^+, in the one-line system. No lines or parentheses are used, since they would imply removal of H. In earlier [10] recommendations 'H_2^+' was added with a vertical line or parentheses, but again (cf. i) the line represented no single bond.

iv) *Deprotonation of Side-Chain Acidic Groups.* The symbols Asp and Glu may have O^- placed at the end of a vertical line above or below them, or in parentheses after them (cf. ii), since O^- replaces the OH removed. Other acidic residues, e.g. Cys, have the charge alone at the end of the vertical line or in parentheses, since the group removed here is H.

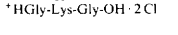
Hence the two ionic forms shown above for a peptide could be drawn as



An isoelectric form of Gly-Lys-Gly could be drawn as



whereas its dihydrochloride could be drawn as



3AA-19.4. *Peptides Substituted at N-2* (see 3AA-16.2 and -17.1)

Glycylnitrosoglycine	NO Gly–Gly or Gly–Gly
Glycylsarcosine (see Appendix)	Me Gly-Sar or Gly–Gly or Gly–Gly
Glycyl-*N*-acetylglycine	Ac Gly–Gly or Gly–Gly
N,N-diglycylglycine	Gly Gly–Gly or Gly–Gly

3AA-19.5. *Cyclic Peptides*

3AA-19.5.1. Homodetic Cyclic Peptides

Cyclic peptides in which the ring consists solely of amino-acid residues in eupeptide linkage may be called homodetic cyclic peptides. Three representations are possible:

i) The sequence is formulated in the usual manner but placed in parentheses and preceded by 'cyclo'. Example: gramicidin S

cyclo(-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-)

or (see 3AA-19.2, sentence 2)

cyclo(-Val-Orn-Leu-DPhe-Pro-Val-Orn-Leu-DPhe-Pro-)

ii) The sequence is again written in one line, but the residues at each end of the line are joined by a lengthened bond, e.g.

Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro

or (3AA-19.2, sentence 2)

Val-Orn-Leu-DPhe-Pro-Val-Orn-Leu-DPhe-Pro

iii) The residues are written on two lines, so that the sequence is reversed on one of them. Hence the CO to NH direction within the peptide bond must be indicated by arrows (3AA-16.2 and -16.3). Hence gramicidin S may by written (using the option of 3AA-19.2, sentence 2):

Val→Orn→Leu→DPhe→Pro
Pro←DPhe←Leu←Orn←Val

3AA-19.5.2. Heterodetic Cyclic Peptides

Heterodetic cyclic peptides are peptides consisting only of amino-acid residues, but the linkages forming the ring are not solely eupeptide bonds; one or more is an isopeptide, disulfide, ester, or other bond.

Their symbolic representation follows logically from that of substituted amino acids (3AA-16.4). Examples:

Oxytocin Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

Cyclic ester of threonylglycylglycylglycine Thr-Gly-Gly-Gly– or (3AA-17.6) H-Thr-Gly-Gly–Gly

3AA-19.6. *Depsipeptides*

Depsipeptides are oligomers formed from amino acids and other bifunctional acids, usually hydroxy acids. They are often cyclic. In symbolic representation, any special symbols used for the hydroxy acids should be defined.

3AA-19.7. *Peptide Analogues*

▲ Analogues of peptides in which the –CO–NH– group that joins residues is replaced by another grouping may be indicated [25] by placing a Greek psi, followed by the replacing group in parenthesis, between the residue symbols where the change occurs. Examples:

Ala-ψ(NH – CO) – Ala for $NH_3^+ - CHMe - NH - CO - CHMe - COO^-$

Ala-ψ(CH = CH, *trans*)-Ala for $NH_3^+ - CHMe - CH = CH - CHMe - COO^-$

3AA-19.8. *Alignment of Peptide and Nucleic-Acid Sequences*

▲ Although hyphens between residues are important in representing peptide sequences (3AA-16), they may be omitted (I) if it is necessary to align sequences with those of nucleic acids; this is an alternative to separating triplets (II):

```
                MetSerIleGlnHis                          Met-Ser-Ile-Gln-His
(I)   AGTATGAGTATTCAACAT        (II)    AGT ATG AGT ATT CAA CAT
      TCATACTCATAAGTTGTA                TCA TAC TCA TAA GTT GTA
```

Part 2, Section B: THE ONE-LETTER SYSTEM (revision and updating of [11])

3AA-20. THE NEED FOR A CONCISE REPRESENTATION OF SEQUENCE

3AA-20.1. *General Considerations Regarding the One-Letter System*

There are difficulties in using the three-letter system (3AA-14 to 3AA-19) in presenting long protein sequences. A one-letter code is much more concise, and is helpful in summarizing large amounts of data, in aligning and comparing homologous

sequences, and in computer techniques for these processes. It may also be used to label residues in three-dimensional pictures of protein molecules.

The possibility of using one-letter symbols was mentioned by Gamow & Yčas [26] in 1958. Šorm *et al.* [27] systematized the idea in 1961 (see, for example, [28]), and Dayhoff and Eck used one-letter symbols derived partly from the code of Šorm *et al.* in their compilations of protein sequences ([29], latest edition [30]). IUB-IUPAC recommendations [11] were approved in 1968 on the basis of proposals of a subcommittee of W. E. Cohn, M. O. Dayhoff, R. V. Eck, and B. Keil, and these recommendations are given here with no substantial change.

3AA-20.2. *Limits of Application of the One-Letter System*

The one-letter system is less easily understood than the three-letter system by those not familiar with it, so it should not be used in simple text or in reporting experimental details of sequence determination. It is therefore recommended for comparisons of long sequences in tables and lists, and in other special uses where brevity is important. If both it and the single-letter system for nucleotide sequences [23] are used in the same paper, particular care should be taken to avoid confusion.

3AA-21. DESCRIPTION OF THE ONE-LETTER SYSTEM

3AA-21.1. *Use of the Code*

The letter written at the left-hand end is that of the amino-acid residue carrying the free amino group, and the letter written at the right-hand end is that of the residue carrying the free carboxyl group. The absence of punctuation beyond either end of a sequence implies that the residue indicated at that end is known to be terminal. A fragmentary sequence is preceded or followed by a slash (/) if its end is not known to be the end of the complete molecule.

3AA-21.2. *The Code Symbols*

The symbols are listed, in alphabetical order of amino-acid names, in Table 1. Table 5 gives them in alphabetical order of symbols.

Note on the Choice of Symbols

Initial letters of the names of the amino acids were chosen where there was no ambiguity. There are six such cases: cysteine, histidine, isoleucine, methionine, serine and valine. All the other amino acids share the initial letters A, G, L, P or T, so arbitrary assignments were made. These letters were assigned to the most frequently occurring and structurally most simple of the amino acids with these initials, alanine (A), glycine (G), leucine (L), proline (P) and threonine (T).

Other assignments were made on the basis of associations that might be helpful in remembering the code, e.g. the phonetic associations of F for *ph*enylalanine and R for *ar*gimine. For tryptophan the double ring of the molecule is associated with the bulky letter W. The letters N and Q were assigned to asparagine and glutamine respectively; D and E to aspartic and glutamic acids respectively. K and Y were chosen for the two remaining amino acids, lysine and tyrosine, because, of the few remaining letters, they were close alphabetically to the initial letters of the names. U and O were avoided because U is easily confused with V in handwritten material, and O with G, Q, C and D in imperfect computer print-outs, and also with zero. J was avoided because it is absent from several languages.

Two other symbols are often necessary in partly determined sequences, so B was assigned to aspartic acid or asparagine when these have not been distinguished; Z was similarly assigned to glutamic acid or glutamine. X means that the identity of an amino acid is undetermined, or that the amino acid is atypical.

Table 5. *The One-Letter Symbols*

One-letter symbol	Three-letter symbol	Amino acid
A	Ala	alanine
B	Asx	aspartic acid or asparagine
C	Cys	cysteine
D	Asp	aspartic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
P	Pro	proline
Q	Gln	glutamine
R	Arg	arginine
S	Ser	serine
T	Thr	threonine
V	Val	valine
W	Trp	tryptophan
X	Xaa	unknown or 'other' amino acid
Y	Tyr	tyrosine
▲ Z	Glx	glutamic acid or glutamine (or substances such as 4-carboxyglutamic acid and 5-oxoproline that yield glutamic acid on acid hydrolysis of peptides)

3AA-21.3. *Spacing*

An important use of the one-letter notation is in presenting alignment of homologous sequences. It is therefore vital not to destroy alignment by variable punctuation or variable width of letters. A single space is therefore left between symbols, as a blank if not occupied by punctuation (3AA-21.4 and 21.5), so that such punctuation can be inserted without destroying alignment. Exactly the same spacing is given to each letter, each blank and each punctuation mark, as in typewritten material or, if printed, as in 'typewriter type font'.

3AA-21.4. *Known Sequences*

A blank between letters indicates that the sequence was determined experimentally. For example, A C D E F G H I K L M N P Q means Ala-Cys-Asp-Glu-Phe-Gly-His-Ile-Lys-Leu-Met-Asn-Pro-Gln

3AA-21.5. *Punctuation in Partly Known Sequences*

Parentheses are used to indicate regions of a sequence in which the composition is known, but the sequence undetermined; they are also placed round the symbol for a single residue to show that its identification is tentative. The one-space symbol ' = ' can be used for ')(' to indicate the end of one unknown sequence and the beginning of another.

If the residue inside parentheses can be positioned with confidence by homology with related proteins, the letters are separated by dots. If their position is arbitrary for lack of even indirect evidence the letters are separated by commas. A slash (/) may be used to separate the symbols for residues that have not been shown experimentally to be corrected, because they are derived from different peptides. A slash before or after a sequence shows that termination has not been demonstrated (3AA-21.1).

This punctuation is illustrated in the comparison of three sequences, where two partly known (a,c) are aligned with a known one (b):

```
a)   (A,C,D)E F G(H.I.K.L=M,N)P Q
b)    R S T E F G H I K L A D P Q
c)    A C D E F/G H I K L(M,N)P Q
```

Thus the sequence of one of the fragments (H.I.K.L) can be inferred with confidence for (a) whereas that of fragments (A,C,D) and (M,N) cannot. Two fragments were sequenced independently in (c). Their positioning is made only by analog with (b).

If more elaborate punctuation is required for special circumstances, it is essential that only one character (or a blank of similar size) should appear between the letters of the code.

Part 3. **Modification of Named Peptides** (a revision and updating of [12])

3AA-22. NAMES AND SYMBOLS FOR DERIVATIVES OF NAMED PEPTIDES

It is often convenient to specify the structure of a peptide by reference to a named sequence of which it is a variant. The recommendations that follow allow this, but they apply only to modifications of the sequence involving normal amide links between residues.

Note. To exemplify any named peptide, the imaginary peptide 'iupaciubin' (to symbolize the harmonious co-operation of IUPAC and IUB), Ala-Lys-Glu-Tyr-Leu, is used in formulating the recommendations below.

3AA-22.1. *Replacement of Residues*

In a peptide of trivial name iupaciubin, if the q^{th} amino-acid residue, starting from the N-terminal end of the chain, is replaced by the amino acid Xaa, the semitrivial name of the modified peptide is [*q*-amino acid]iupaciubin, and the abbreviated form is [Xaa^{q}]iupaciubin. A designation of the chain may be placed before the residue number, e.g. [Ala^{B12}]insulin(cattle) (see Comment f). Examples:

[8-Citrulline]vasopressin, [Cit^{8}]vasopressin;

[5-Isoleucine,7-alanine]angiotensin II, [Ile^{5},Ala^{7}]angiotensin II.

Notes

a) In the full name, the replacement amino acid is designated by its residue name, not the name of its acyl group (e.g. glycine, not glycyl). This name, and the position of replacement, are given in square brackets.

b) In the abbreviated form, the amino-acid residues are designated by standard 3-letter symbols (Table 1), the first letter only being a capital (3AA-14), in square brackets.

c) In the abbreviated form the position of substitution is indicated in a special fashion, i.e. by a superior numeral, to indicate that it is a residue, not an individual atom, that is being replaced.

d) The residue replaced is not designated in these semitrivial names in order to keep the names short, and because this form of nomenclature of 3AA-22.1 clearly differs from ordinary substitution nomenclature.

e) The replacement of an amino-acid residue by its enantiomer may be shown by application of this rule as follows: the
▲ replacement in iupaciubin of L-tyrosine at position 4 by D-tyrosine results in [4-D-tyrosine]iupaciubin with the abbreviations [D-Tyr^{4}]iupaciubin. A mixture of this with iupaciubin gives [4-*ambo*-tyrosine]iupaciubin or [*ambo*-Tyr^{4}]iupaciubin (3AA-13.2 and 3AA-19.2). Examples: [D-Ser^{1}]corticotropin; [D-Asp^{1}]angiotensin II.

▲ f) Specification of a sequence may require the species as well as the peptide to be named. If so, the name of the species should be attached, in parenthesis, to the name of the peptide whenever a modifying prefix is present. Thus a substitution in cattle insulin could give [Ala^{B12}]insulin(cattle). (We prefer 'cattle' as the adjective for this species, since 'bovine' is not used in common speech to designate the species, but to compare human attributes with those of the cow, and 'ox' can be misleading.)

▲ g) It may be convenient to represent replacement of Gln by Glu or of Asn by Asp with the prefix 'desamido'. Thus [Glu^{30}]corticotropin(pig) could be called desamido^{30}-corticotropin(pig). Similarly replacement of Gla by Glu can be designated with the prefix 'decarboxy'.

3AA-22.2. *Extension of the Peptide Chain*

The compounds obtained by extension of a peptide at either the N-terminus on the C-terminus are designated by the kinds of names and abbreviations shown below; these are in accordance with general principles of peptide nomenclature (3AA-13.1). Examples:

a) Extension at the N-terminus	
Aminoacyliupaciubin	Xaa-iupaciubin
Valyliupaciubin	Val-iupaciubin
Valylglycyliupaciubin (for extension by two residues)	Val-Gly-iupaciubin
b) Extension at the C-terminus	
Iupaciubinylamino acid	Iupaciubinyl-Xaa
Iupaciubinylleucine	Iupaciubinyl-Leu

▲ This rule is not directly applicable to extension at the C-terminus of natural peptides that possess a terminal amide group, such as oxytocin and α-melanotropin. For these, a new name should be given to the corresponding peptide with a free carboxyl group by adding 'oic acid' to the trivial name, e.g. oxytocinoic acid from oxytocin, so that extension can then be denoted as above, e.g. oxytocinoyl-Xaa.

Note

The enkephalins are the two peptides Tyr-Gly-Gly-Phe-Leu and Tyr-Gly-Gly-Phe-Met. Designations such as Leu-, leucyl- and leucine-enkephalin have been given to the former, with corresponding terms for the latter. These all wrongly imply N-terminal extension, and could not be used together with any indication of such extension. Morley [25] has advocated [Leu]enkephalin, if necessary [Leu^5]enkephalin, in accordance with 3AA-22.1, implying that enkephalin means Tyr-Gly-Gly-Phe-Xaa. We believe that [Leu^5]enkephalin is the best designation.

3AA-22.3. *Insertion of Residues*

▲ The compound obtained by insertion of an additional amino-acid residue Xaa in the position between the q^{th} and the $(q+1)^{th}$ residue of the peptide iupaciubin is named endo-*q*a-amino acid-iupaciubin (abbreviated form: endo-Xaa^{qa}-iupaciubin). Example:

endo-4a-tyrosine-angiotensin II, endo-Tyr^{4a}-angiotensin II.

Notes

a) This form has analogies in other fields where endo implies the insertion of something into a structure (e.g. endo-methylene). The prefix or index *q*a is based on analogies with the steroids where the atoms inserted into a ring after atom no. *q* are designated *q*a, *q*b, etc.

b) The prefix 'homo' is not suitable for designating the insertion of a whole residue, especially since it is commonly used to modify the names of individual amino acids, e.g. homoserine (3AA-2.3).

c) Multiple insertions, and insertion of two or more residues together in the same place in the chain, are shown by a logical extension of this recommendation. Thus the insertion into the peptide iupaciubin of threonine between residues 1 and 2, and of valine and glycine (in that order) between residues 4 and 5, is shown by the name 'endo-1a-threonine,4a-valine,4b-glycine-iupaciubin' and the abbreviation 'endo-Thr^{1a},Val^{4a},Gly^{4b}-iupaciubin'.

3AA-22.4. *Removal of Residues*

The compound obtained by the formal removal of an amino-acid residue from the peptide iupaciubin in position *q* is designated by the name des-*q*-amino acid-iupaciubin, abbreviated des-Xaa^{q}-iupaciubin. Example:

des-7-proline-oxytocin, des-Pro^7-oxytocin

Notes

a) Removal of a whole residue is indicated in a way similar to that for removal of a ring in steroids, e.g. des-*A*-androstane.

b) The form 'de' is not suitable as a prefix because it is easily confused, in speaking, with D (for configuration).

▲ c) Multiple deletions are designated similarly, e.g. des-Ile^3,Asn^5-oxytocin. If a complete sequence is to be removed, the first and last loci of this sequence are all that need be specified, and they should be put in parentheses with a hyphen between them, e.g. des-(B24-B28)-insulin(mouse).

3AA-22.5. *Substitution of Side Chains of Residues*

The compound formed by introducing an additional amino-acid residue as a substituent of the side chain of a residue in a peptide is named by applying the rules of peptide nomenclature (3AA-7, -9 and -13) to the trivial name, as follows.

22.5.1. Acylation of a Side-Chain Amino Group

If the additional residue acylates an amino group of a peptide, the name of the additional amino acid is written, in its acyl group form (3AA-9.3), and prefixed by symbols indicating the position of substitution (atom and residue number, see 3AA-13.4).

Example: the imaginary compound

Val—┐
Ala-Lys-Glu-Tyr-Leu

derived by acylation of the ε-amino group of the lysine residue at position 2 of iupaciubin (Ala-Lys-Glu-Tyr-Leu) with a valyl group is named $N^{\varepsilon 2}$-valyl-iupaciubin (abbreviated $N^{\varepsilon 2}$-Val-iupaciubin).

22.5.2. Other Substituents Named as Prefixes

Other substituents that can be named as prefixes are treated similarly to amino acyl groups. Examples:

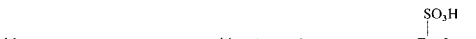


$N^{6.2}$-Methyliupaciubin for Ala-Lys-Glu-Tyr-Leu



$C^{4.3}$-Carboxyiupaciubin for Ala-Lys-Glu-Tyr-Leu

(alternative to replacement with Gla under 3AA-22.1)



$O^{4.4}$-Sulfoiupaciubin or iupaciubin $O^{4.4}$-sulfate for Ala-Lys-Glu-Tyr-Leu

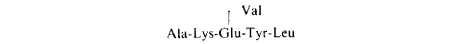
$O^{4.12}$-Sulfogastrin or gastrin $O^{4.12}$-sulfate for gastrin II.

$N^{\varepsilon B1}$,$N^{\varepsilon B29}$-bis(Boc)insulin or $N^{2.B1}$,$N^{6.B29}$-bis(Boc)insulin

22.5.3. Acylation by a Side-Chain Carboxyl Group

If an additional amino acid is joined by amide formation between its 2-amino group and the side-chain carboxyl of the peptide iupaciubin, so that the additional amino acid has a free 1-carboxyl group, the derivative is named by specifying the position of substitution (atom and residue number, 3AA-13.4) and it is given the designation 'iupaciubinyl-amino acid'.

Example: the imaginary compound



in which valine is acylated by the γ-carboxyl group of a glutamic residue in position 3 (Glu-3) of iupaciubin (Ala-Lys-Glu-Tyr-Leu), is named *N*-(iupaciubin-$C^{\delta 3}$-yl)valine, or *N*-(iupaciubin-$C^{5.3}$-yl)valine, abbreviated to iupaciubin-$C^{5.3}$-yl-Val.

Prefixes may also need to be formed from peptide names for use as substituents in other types of compound, as described in 3AA-13.5.

3AA-22.6. *Partial Sequences* (fragments)

▲ A peptide derived from a named peptide iupaciubin by removal of all residues before the p^{th} and all after the q^{th} is named as iupaciubin-(*p*-*q*)-peptide. Examples:

From α-melanotropin

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2
1 2 3 4 5 6 7 8 9 10 11 12 13

we may have

Met-Glu-His-Phe-Arg-Trp-Gly or α-melanotropin-(4-10)-peptide
4 10

or, to illustrate the naming of a peptide that contains two fragments, and also a C-terminal amide group:

His-Phe-Arg-Lys-Pro-Val-NH_2 may be called α-melanotropin-(6-8)-(11-13)-peptide amide.

For oligopeptides it may be convenient to state the length of peptide, e.g. iupaciubin-(2-4)-tripeptide, but this is not normally useful for peptides of over about twelve residues, because the larger multiplying affixes are not widely known. Such a check on the number of residues is particularly useful when two or more sequences are joined, e.g. α-melanotropin-(6-8)-(11-13)-hexapeptide amide.

3AA-22.7. *Peptides with Reversed Sequence and Enantiomers*

The peptide whose sequence is the reverse of a named peptide may itself be named with the prefix '*retro-*', giving *retro*-iupaciubin from iupaciubin. The enantiomer of a named peptide may be specified with the prefix '*ent-*' (a contracted form of *enantio-*, F-6.4 of [15]), giving *ent*-iupaciubin from iupaciubin.

3AA-22.8. *Peptide Analogues*

▲ Analogues of peptides in which the –CO–NH– group that connects two residues is replaced by another group (3AA-19.7) may be indicated by placing ψ in square brackets before the name [25]. The ψ is placed between superscripts indicating the residues modified, and is followed by a comma and the replacing group, e.g. [$^{3}\psi^{4}$,CH_2-S]iupaciubin.

3AA-22.9. *Summary of Modification Nomenclature*

The application of the principles of 3AA-22 is illustrated in Table 6.

Table 6. *Application of the methods of modifying named peptides*

Section	Operation	Short name	Structure
	—	Iupaciubin	$\overset{1}{\text{Ala}}\text{-}\overset{2}{\text{Lys}}\text{-}\overset{3}{\text{Glu}}\text{-}\overset{4}{\text{Tyr}}\text{-}\overset{5}{\text{Leu}}$
22.1	Replacement	[Phe^4]iupaciubin*	Ala-Lys-Glu-$\overset{4}{\text{Phe}}$-Leu
22.2a	Extension (N-terminal)	Arg-iupaciubin	$\overset{1}{\text{Arg}}$-Ala-Lys-Glu-Tyr-$\overset{5}{\text{Leu}}$
22.2b	Extension (C-terminal)	Iupaciubinyl-Met	Ala-Lys-Glu-Tyr-Leu-Met
22.3	Insertion	Endo-Thr^{2a}-iupaciubin	Ala-Lys-$\overset{2}{\text{Thr}}\overset{2a}{}\overset{3}{}$-Glu-Tyr-Leu
22.4	Removal	Des-Glu^3-iupaciubin	Ala-$\overset{2}{\text{Lys}}$-$\overset{4}{\text{Tyr}}$-Leu
22.5.1	Side-chain substitution on amino group	$N^{\varepsilon 2}$-Val-iupaciubin	Ala-Lys-Glu-Tyr-Leu (Val attached to Lys)
22.5.2	Side-chain substitution on carboxyl group	$C^{\delta 3}$-Iupaciubinyl-Val	Ala-Lys-Glu-Tyr-Leu (Val attached to Glu)
22.6	Partial sequence	Iupaciubin-(2-4)-peptide	Lys-Glu-Tyr
22.7	Reversal of sequence	*retro*-Iupaciubin	Leu-Tyr-Glu-Lys-Ala
22.7	Enantiomer	*ent*-Iupaciubin	D-Ala-D-Lys-D-Glu-D-Tyr-D-Leu
22.8	Replacement of CO–NH of peptide bond	[$^3\psi^4$,CH_2–S]iupaciubin	Ala-Lys-Glu-$\psi(CH_2\text{-}S)$-Tyr-Leu

* Square brackets are required to indicate replacement, but are not used for most other modifications.

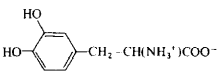
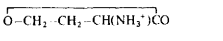
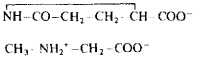
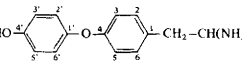
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Appendix. **Amino Acids with Trivial Names** (excluding those listed in Table 1)

It is often helpful to use trivial names in order to avoid cumbersome systematic or semisystematic names, particularly if the substance has to be named frequently. Coining of trivial names is treated in 3AA-2.1, and a number of existing trivial names are listed in the appendices to the previous edition of recommendations on amino-acid nomenclature [6]; only the commoner are listed here.

Trivial name	Symbol	Structure of substance or of derived ion in the form predominating at neutral pH
β-Alanine	βAla	$NH_3^+-CH_2-CH_2-COO^-$
Allysine		$HCO-[CH_2]_3-CH(NH_3^+)COO^-$
Citrulline	Cit	$NH_2-CO-NH-[CH_2]_3-CH(NH_3^+)COO^-$
Cystathionine	Ala | Hcy	$CH_2-CH(NH_3^+)COO^-$ | $S-[CH_2]_2-CH(NH_3^+)COO^-$
Cysteic acid	Cya	$^-O_3S-CH_2-CH(NH_3^+)COO^-$
Cystine	Cys | Cys	$S-CH_2-CH(NH_3^+)COO^-$ | $S-CH_2-CH(NH_3^+)COO^-$
Dopa		
Homocysteine	Hcy	$HS-CH_2-CH_2-CH(NH_3^+)COO^-$
Homoserine	Hse	$HO-CH_2-CH_2-CH(NH_3^+)COO^-$
Homoserine lactone	Hsl	
Lanthionine	Ala | Cys	$CH_2-CH(NH_3^+)COO^-$ | $S-CH_2CH(NH_3^+)COO^-$
Ornithine	Orn	$NH_3^+-[CH_2]_3-CH(NH_3^+)COO^-$
5-Oxoproline	Glp	
Sarcosine	Sar	$CH_3-NH_2^+-CH_2-COO^-$
Thyronine		
Thyroxine	Thx	