1
Amino Acids

BY G. C. BARRETT

1 Introduction

The occurrence, chemistry and analysis of amino acids contained in the literature of 1987 are reviewed in this Chapter, which is arranged in the sections as used in all previous Volumes in this Specialist Periodical Report.

Access to the literature for creating this Chapter has been by way of Chemical Abstracts (to Volume 108, issue 9) and Biological Abstracts (to issue 8 of Volume 85), supplemented by scanning a selection of major journals so as to cover the 1987 literature adequately. The abstracts coverage also nets a few citations published in 1986, and these are included to give continuity for the topic over the years.

2 Textbooks and Reviews

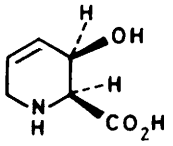
Uses of amino acids and simple derivatives in synthesis are surveyed in recent texts.[1,2] Reviews of every conceivable amino acid with a sulphur functional group in the side chain comprise a complete Volume of Methods in Enzymology.[3] A Symposium has covered roles of amino acids in various disorders.[4] A similarly thorough treatment has been given to the biosynthesis of protein amino acids.[5]

Other textbooks and reviews are located in the relevant sections of this Chapter.

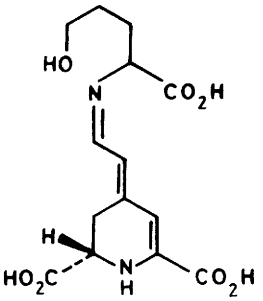
3 Naturally Occurring Amino Acids

3.1 Occurrence of Known Amino Acids.- Amino acids in the Murchison meteorite show unusually high abundance of ^{2}H,[6] ^{15}N,[6] and ^{13}C,[7] giving further evidence for extraterrestrial origins for these amino acids (as opposed to contamination of the meteorite after arrival).

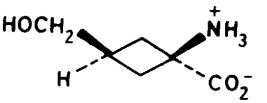
Amino acids and peptides in algae have been reviewed.[8] Bacterial sources of less common amino acids as protein constituents (phycobiliproteins[9,10]) include Mastigocladus laminosus and Calothrix (γ-N-methyl asparagine),[9] and Chromatium



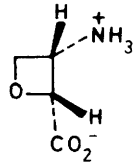
(1)



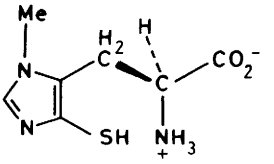
(2)



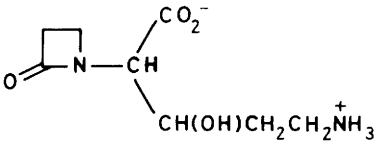
(3)



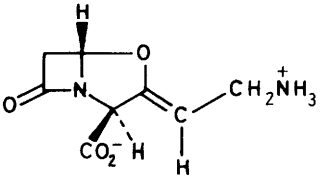
(4)



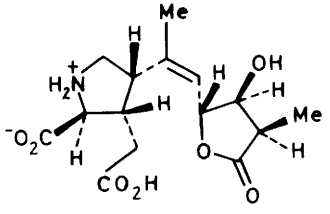
(5)



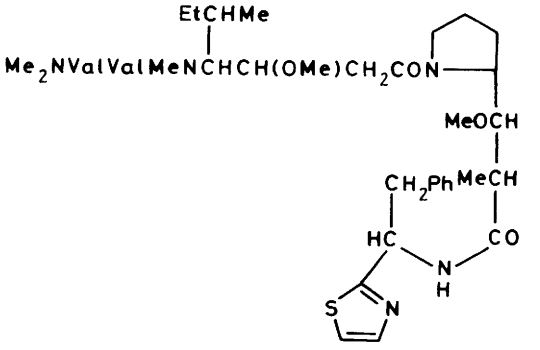
(6)



(7)



(8)



(9)

vinosum (N^{ε},N^{ε}-dimethyl lysine).[11] p-Aminophenylalanine occurs in Vigna, as a growth inhibitor of Escherichia coli.[12]

Plant sources include tulip, with 4-methyleneglutamine identified as one constituent of its leaves.[13] Trees of the Copaifera genus, whose leaves contain N-methyl trans-4-hydroxyproline in substantial amounts (up to 3% dry weight and representing 10% of the nitrogen content), are thought to be protected from bruchid beetle larval attack by this amino acid.[14] The same amino acid occurs in Melaleuca, together with the corresponding betaine.[15]

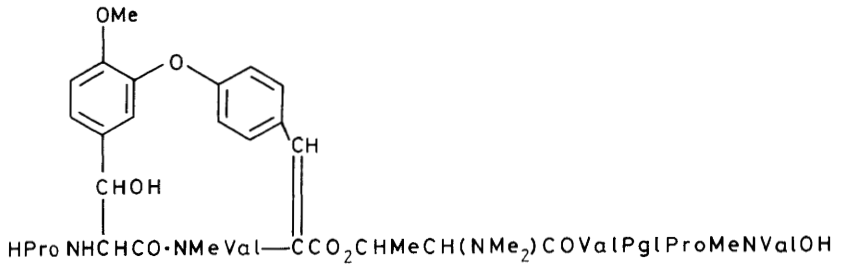
The free D-alanine content of bivalves is surprisingly high, frequently far exceeding that of its L-enantiomer. Several bivalve species also contain D-aspartic acid in concentrations approaching that of the L-isomer.[16] The absence of D-valine[16] in these animals must have significance that has not yet been a source of speculation.

The accumulation of D-arginine in rat liver mitochondria has been reported.[17] Another notable occurrence is of β-hydroxyasparagine (not previously known as a protein constituent), and β-hydroxyaspartic acid, in Vitamin K-dependent proteins[18] and in bovine low – density lipoprotein receptor and bovine thrombomodulin.[19] The introduction of isodityrosine crosslinking residues in extensin resulting from oxidative coupling in vivo cannot be repeated in vitro using H_2O_2 and peroxidase, which introduces dityrosine crosslinks instead into this protein.[20] N^{π}-Methyl histidine has been identified for the first time as a protein constituent (in rabbit skeletal-muscle light-chain kinase).[21]

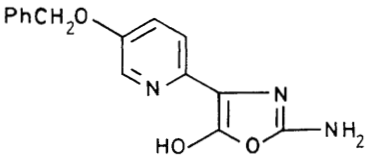
3.2 New Natural Amino Acids.- The presence of L-2-amino-4-chloropent-4-enoic acid in fruit bodies of Amanita pseudoporphyria Hongo accounts for antibacterial properties of this fungus;[22] Amanita abrupta contains (2S,4Z)-2-amino-5-chloro-6-hydroxy-4-hexenoic acid (as well as three other unusual but previously known amino acids).[23] (2S,3R)-(-)-3-Hydroxybaikiain (1) is a constituent of the toxic mushroom Russula subnigricans Hongo.[24]

Fruits of Rivina humilis contain the new betalain (2), a 5-hydroxy-norvaline derivative.[25] Smaller ring moieties appear in cis-1-amino-3-hydroxymethyl-cyclobutane-1-carboxylic acid (3), found in Atelia herbert smithii Pittier (Sophoreae, Leguminoseae),[26] and (2R,3S)-oxetin (4).[27,28] L-Ovothiol A and its N,N-dimethyl analogue (L-ovothiol B) have been proved to possess structure (5)by virtue of its synthesis from (12).[29] The authors suggest that previously described marine 3-mercaptohistidines should be revised to the 1-methyl structures.[29] The novel hydroxyornithine derivative proclavaminic acid (6) from the mycelium of the clavulanic acid-producing organism Streptomyces clavuligerus ATCC 27064 undergoes enzymatic cyclization in cell-free extracts to clavaminic acid (7).[30]

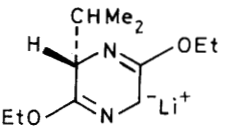
A further example of the well populated class of natural N-carboxyalkyl amino



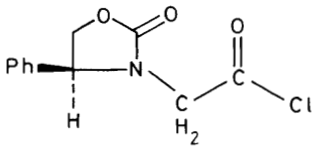
(10)



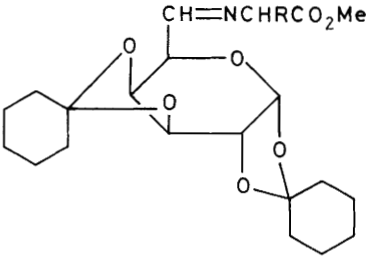
(11)



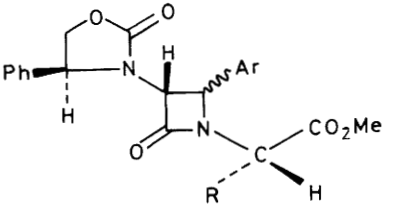
(12)



(14)

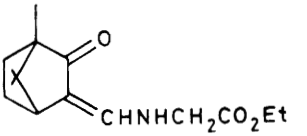


(13)

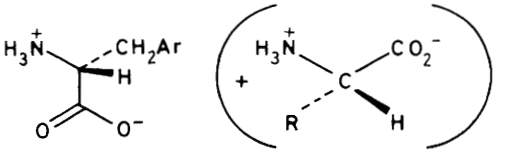


(15)

(i) NaOH(aq) | (iii) Birch reduction
(ii) H_2/Pd | (iv) hydrolysis



(16)



acids is (2S,7S)-N^6-(1-carboxyethyl)ornithine, from Streptococcus lactis.[31]

More complete information is now available[32] on new amino acids from the red alga Chondria armata, previously noted in this Specialist Periodical Report (Vol.19, p.2) to contain seven new amino acids. However, only domoilactone A (8; a correction of the structure given in Vol.19, p.2) and domiolactone B (epimer of A at the chiral centre carrying the hydroxy group) have been described so far.[31,32]

3.3 New Amino Acids from Hydrolysates. - The earlier section (3.1) included reports on the occurrence of known amino acids as noteworthy protein constituents. This section develops the same topic but with new and therefore even more noteworthy analogues.

Bovine ligament elastin contains a cross-linking amino acid related to isodesmosine, insofar as it is a pyridinium compound, but it also carries a C=C bond in an extra side chain.[33] It has been christened "pentasine", as it is derived from the condensation of five lysine residues.

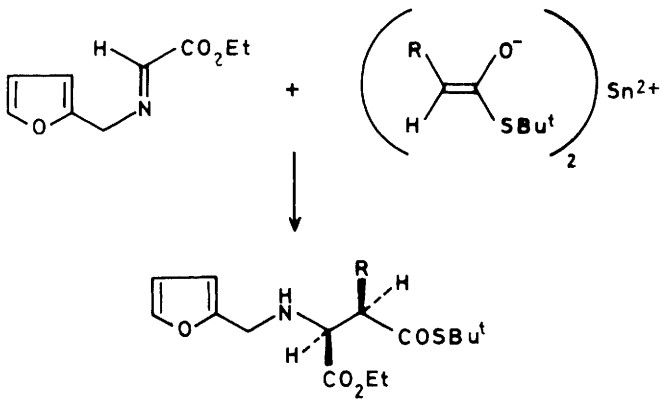
Fifteen years' work leads to the structure assigned (without stereochemical details) to dolastatin 10 (9).[34] It is a "pentapeptide" from the sea hare Dolabella auricularia and is the most potent antineoplastic compound known, containing four amino acids not previously known in Nature.[34] A corrected abstract has been published[35] for an unusual peptide from a strain of Streptomyces AM-2504 that contains a β-hydroxydopa derivative (mild hydrolysis of AM-2504 gives (10) and an amino acid of relative molecular mass 141).

The vapour-phase hydrolysis regime for peptides and proteins, employing 7M hydrochloric acid containing 10% trifluoroacetic acid at 150° during 22 - 45 minutes, will be of considerable interest.[36]

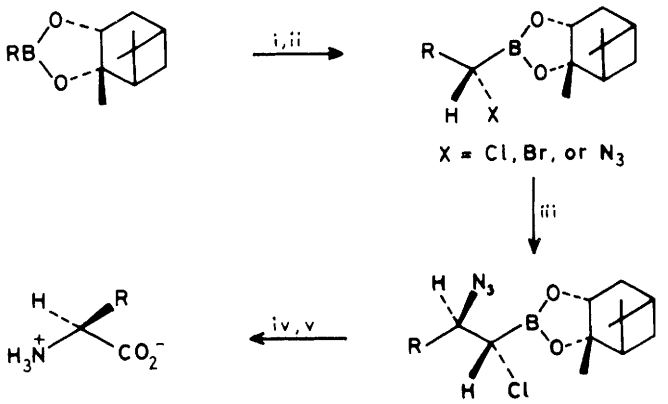
4 Chemical Synthesis and Resolution of Amino Acids

4.1 General Methods. - A broad review of amino acid synthesis [37] and a more limited coverage of contributions to synthesis of unusual natural amino acids [38] have appeared. Formation of N-acylamino acids by the amidocarbonylation of aldehydes (CO/amide/homogeneous mixed-metal catalyst systems)[39] has been extensively reviewed.[40]

This section is divided into applications, either of well established or of lesser-known general methods. Later sections include several examples of variations of standard general methods. In the former category, the Bucherer-Bergs synthesis has been used for the synthesis of α-(5-hydroxy-2-pyridyl)glycine starting from (5-benzyloxy)pyridine-2-carboxaldehyde, though with the unexpected intermediacy of the oxazole (11),[42] rather than the usual hydantoin. Treatment of (11) with 2M sodium hydroxide at 120° during 8 hours gave the required product. Other hydantoin-based syntheses include carboxylation of α-aminonitriles

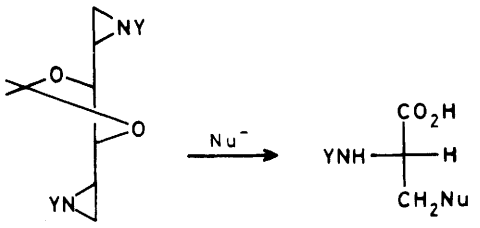


Scheme 1



Reagents: i, $LiCHCl_2$ or $LiCHBr_2$; ii, NaN_3; iii, $LiCHCl_2$; iv, $NaClO_2$; v, H_2/Pt or Pd

Scheme 2



(17) (18)

(via carbamate, oxazolidinone, and α-isocyanato-acid amide).[42]

The Strecker synthesis has been applied to the preparation of α-hydroxymethyl-α-(3,4-disubstituted phenyl)glycines.[43] The acetamidomalonate synthesis continues to be widely used in all its variations, e.g. for 3-(3-pyridyl)- and 3-(3-benzo[b]thienyl)-D-alanines (including enzymic resolution),[44] and for 3-(2-carboxy-4-pyridyl)- and 3-(6-carboxy-3-pyridyl)alanines.[45]

Alkylation of glycine derivatives has also become widely used in its many variants. The predominance of the route based on alkylation of glycine Schiff bases continues, to which novel routes occasionally arise (e.g. to t-butyl *N*-(t-butyloxycarbonyl)iminoacetate from $BocNHCHBrCO_2{}^tBu$, this being alkylated with either a Grignard reagent, or an enamine, or morpholinostyrene[46]). 6-Fluorodopa has been prepared from veratric acid (via the derived benzylic bromide) and $Ph_2C{=}NCH_2CO_2Et$.[47] Similar alkylations of *N*,*N*-dibenzylglycine esters,[48] methyl nitro-acetate,[49] and reduction of α-diazo-acetoacetates $RCOC(N_2)CO_2Et$ with H_2/Pd in acetic acid to give α-acetamidoacetoacetates [50] further illustrate applications of established methods.

Tin(II) enolates of thiolesters undergo diastereoselective addition to *N*-furfuryl imines (Scheme 1).[51] Another example of a novel route that might develop into a useful general method involves photochemical addition of N_3CO_2Et to silylenols $R^1R^2C{=}C(OR^3)OSiMe_3$, giving *N*-(ethoxycarbonyl)amino acid esters in 45 - 75% yields.[52]

4.2 Asymmetric Synthesis.- Some of the general methods in the amino acid field offer useful enantiospecific synthesis opportunities in the α-amino acid area. The later Section 4.17 deals both with general methods and asymmetric synthesis of β- and higher homologous amino acids. The requirement for enantiomerically pure α-amino acids in biological and synthetic studies has sustained the development of stereoselective synthetic methods, frequently feeding back improvements into established general methods.

The "bis-lactim ether" method, introduced by Schöllkopf and his co-workers, continues to be used profitably for asymmetric synthesis of α-amino acids. Typically, L-Ovothiol A (5) and its *N*,*N*-dimethyl analogue (L-ovothiol B) have been prepared by this method, from (12).[29] The method involves alkylation of the chiral carbanion of (12), commonly using an alkyl halide as electrophile[29] [as in a synthesis of enantiomers of phosphinothricin, $MeP(O)(OH)CH_2CH_2CH(NH_3{}^+)CO_2{}^-$[53]]. However, Michael additions (better than 99% stereoselectivity with methyl 2-alkenoates[54]), synthesis of α-amino-γ-nitroalkanoates using nitro-alkenes,[55] and "non-Michael additions" [bis-lactim ether (12) - $Ti(NEt_2)_3$ derivatives add diastereoselectively to $R^2CH{=}CR^1CHO$ to give (2*R*,3*S*)-$R^2CH{=}CR^1CH(OH)CH(NH_3{}^+)CO_2{}^-$[56]] have been illustrated. Alkylation by oxiranes[57] has been used for synthesis of 2-methoxyethoxymethyl-protected (*R*)-homoserine methyl esters. α-Methylated cyclic amino acids have been synthesised

by this method.[58]

The bis-lactim ether method is a "hidden" form of Schiff base alkylation, a widely used approach with a longer history. This is represented in the recent literature with alkylations of glycine chiral Schiff bases, giving proline homologues through alkylation with $I(CH_2)_nHal$,[59] or α-alkyl-α-amino acids using the D-galactodi-aldehyde imines (13) with from 23 to >95% asymmetric induction.[60] The "Evans - Sjoegren" ketene derived from the glycyl chloride (14) gives β-lactams by cycloaddition to benzylidene-amino acids.[61] On alkaline hydrolysis and hydrogenolysis (H_2/Pd), the adduct (15) gives optically pure amino acids. Michael addition of the (S)-(o)-[(N-benzylprolyl)amino]benzophenone-derived Schiff base of glycine to acrylates gives L-glutamic acid (and acrolein gives L-proline) after cleavage using aqueous hydrochloric acid, with almost complete diastereoselection.[62] A related method uses a chiral alkylating agent, e.g. alkyl sulphates of D-glucose (0-76% enantioselection).[63]

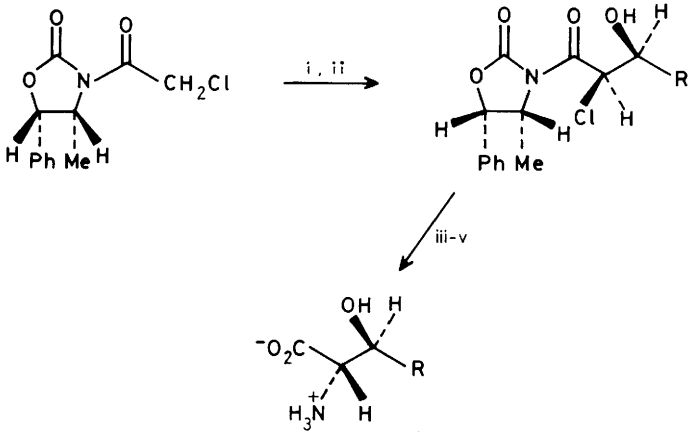
Alkylation of the imine (16) from 3-hydroxymethylenecamphor and ethyl glycinate with alkyl chlorides after carbanion formation with lithium di-isopropylamide gives only low to moderate enantiomer excesses; better diastereoselectivity is seen for sarcosine analogues.[64]

The Schiff base from β-D-galactopyranosylamine is a source of D-amino acids through diastereoselectivity of Strecker reactions based on it.[65] Aldehydes react with the tetra-O-pivaloyl derivative catalyzed by Me_3SiCN and $ZnCl_2$ or $SnCl_4$ to give diastereoisomer mixtures favouring the R-epimer by 7-13:1.[65]

Introduction of the nitrogen function diastereoselectively is a feature of well established aminolysis procedures. Serine β-lactone prepared from protected serines by the Mitsunobu method reacts with organometallic reagents, R_2CuLi or $R_2Cu(CN)Li_2$, to give optically pure β-hydroxyamino acids resulting from alkylation of the original serine methylene group.[66] Lesser enantioselectivity is found for the lactone derived from N-benzylserine; conversely, optical purity better than 99.4% is achieved through copper(I)-catalyzed Grignard alkylation.[66] Reductive aminolysis of oxazolinones with (S)-(-)-phenylethylamine and $H_2/PdCl_2$ gives corresponding (S)-amino acid derivatives.[67] Azidolysis of (S)-1-halogenoalkylboronates of (S)-pinanediol is followed by generation of the carboxy group in the target L-amino acids by a novel method involving insertion of a chloromethylene group through reaction with $LiCHCl_2$ (Scheme 2).[68]

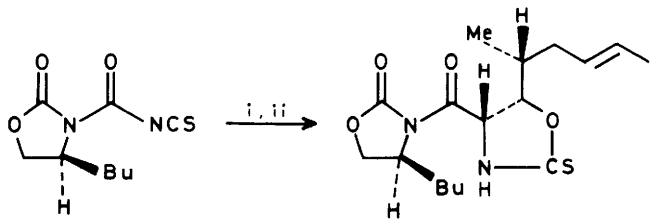
Chiral bis-aziridines obtained from D-mannitol can undergo nucleophilic ring-opening in one of two ways; it has been found that the pathway leading to (S)-amino acids is followed [(17)→(18)] when R_2CuLi is used.[69]

Alkylation of chiral oxazolidinones or imidazolinones has been developed further from its initial exploration as a route to α-amino-β-hydroxy acids.[70-73] The hydroxy group originates in alkylation by a carbonyl compound - i.e. a diastereoselective aldol condensation in its original form[70] - and complete stereoselectivity leading to the anti-relationship of the hydroxy and amino groups



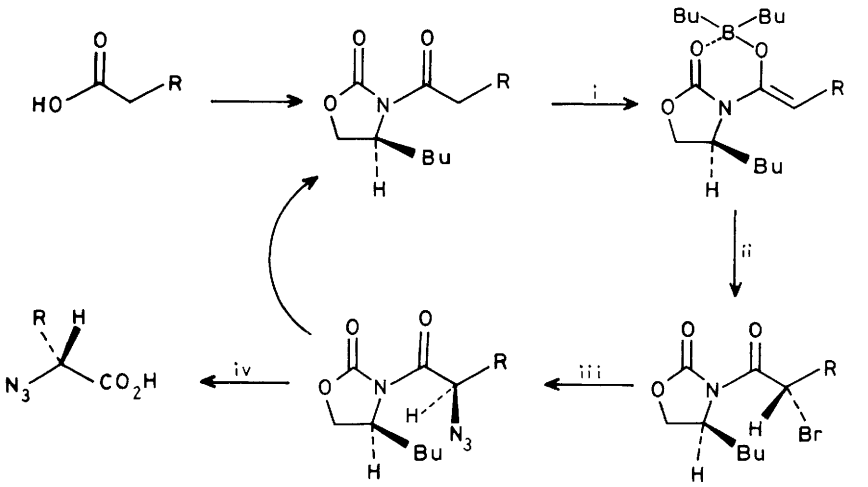
Reagents: i, Bu_2BOTf/NEt_3; ii, RCHO; iii, $NaN_3/DMSO$; iv, OH^-; v, $H_2/Pd—C$

Scheme 3



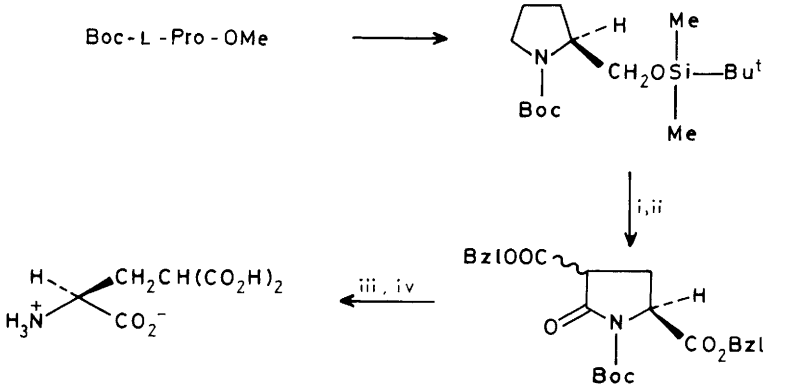
Reagents: i, $Sn(OTf)_2$; ii (R)—OHC—$CHMeCH_2CH$=$CHMe$

Scheme 4



Reagents: i, Bu_2BOTf, R_3N; ii, NBS; iii, NaN_3; iv, LiOH

Scheme 5



Reagents: i, RuO_4; ii, base, ZCl; iii, H_3O^+; iv, H_2/Pd

Scheme 6

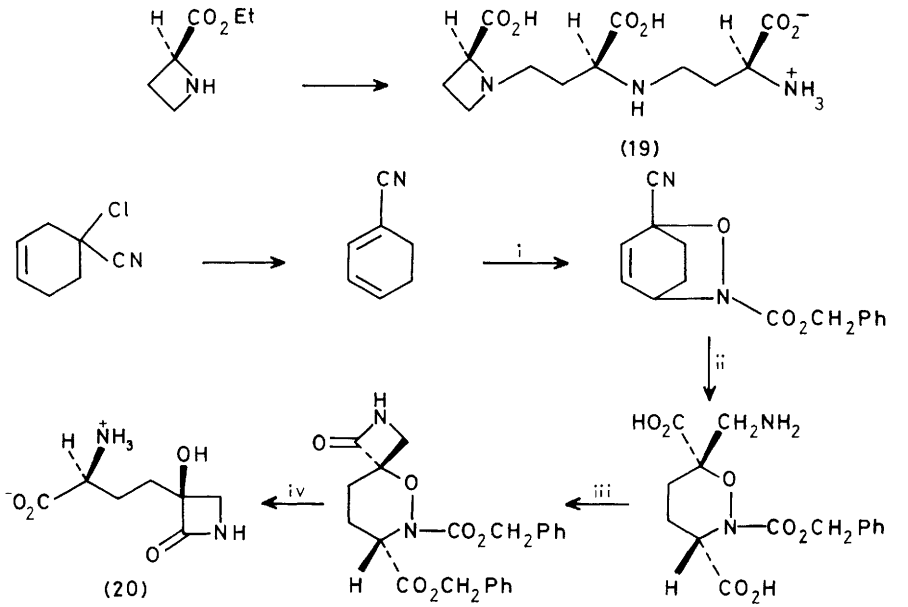
calls for a longer route (Scheme 3).[71] This variation has been used[72] in alternative approaches to the synthesis of unusual constituents of the peptide Echinocandin D (aldolization of the starting material in Scheme 4 with (R)-2-methyl-4-hexenal and tin(II) triflate).[72] [Both these amino acids were synthesized by Ohfune in 1986 (Vol.19, p.10).] Chiral N-acyloxazolidinones, after conversion into dibutyl boron enolates, undergo successive diastereoselective bromination (N-bromosuccinimide) and azidolysis, offering a general asymmetric synthesis (e.e. better than 98%) suitable for multifunctional amino acids (Scheme 5) and involving recovery of the chiral auxiliary.[73]

There is a regular supply of papers describing asymmetric hydrogenation of acetamidocinnamic acid (82-93% enantiomeric excess using chiral Rh or Re complexes as homogeneous catalysts)[74] and its close relatives.[75] Regular hydrogenation systems lead to rather low enantiomeric excesses when operating on unsaturated amino acid (S)-phenylethylamides [5-cyano-2-(hydroxyimino)valeric acid gives the D-lysine derivative in 6-12% diastereoisomeric excess with H_2/Pt[76]] or on 2-trifluoromethyl-4-alkylidene oxazolinones (H_2/Pt/(S)-phenylethylamine).[77]

A brief reference to enzyme-catalyzed syntheses of L-amino acids is usually located in this Chapter in the later section covering protein amino acids, but unusual enzymic methods are found a place here. The availability of relatively large quantities of cloned E.coli aspartate transaminase for mediating the conversion of α-keto-acids into corresponding α-amino acids offers a practical route to a wide range of aliphatic and aromatic side chains. The essential role of large relative amounts of enzyme in these asymmetric syntheses and use of aspartic or glutamic acids as nitrogen source are notable aspects of this unselective application of an enzyme.[78] Dimethyl meso-N-benzylpyrrolidine-2,5-dicarboxylate gives N-benzyl-D-proline methyl ester through selective hydrolysis catalyzed by pig liver esterase, followed by radical decarboxylation of the N-hydroxypyridine-2-thione ester.[79]

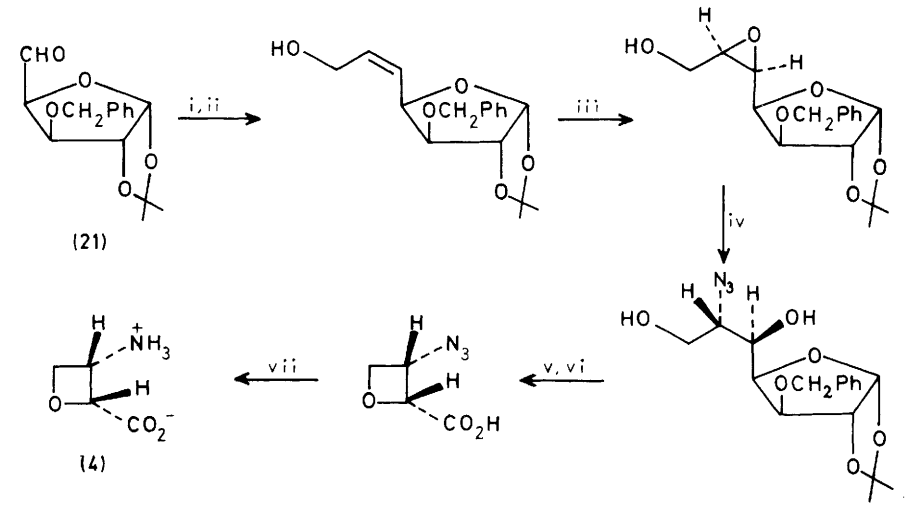
4.3 Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids. - The protein amino acids feature incidentally in exploration and development of new synthetic methods (see preceding sections). Many of the reactions are covered under Section 6.3 'Specific reactions of amino acids' with side-chain modifications, and often amount to the synthesis of one protein amino acid from another. Having drawn attention to these other locations, the main interest as far as this Section is concerned lies in developments in enzymatic and bacterial methods applicable to large-scale production of protein amino acids. However, limitations of space permit only representative citations of these papers (readers are directed inter alia to Section 16 'Fermentation and Bio-industrial Chemistry' in Chemical Abstracts for more complete access to this literature). Biosynthetic studies for protein amino acids are otherwise excluded.

Several reviews[80] have appeared, dealing with low-cost enzymatic synthesis of



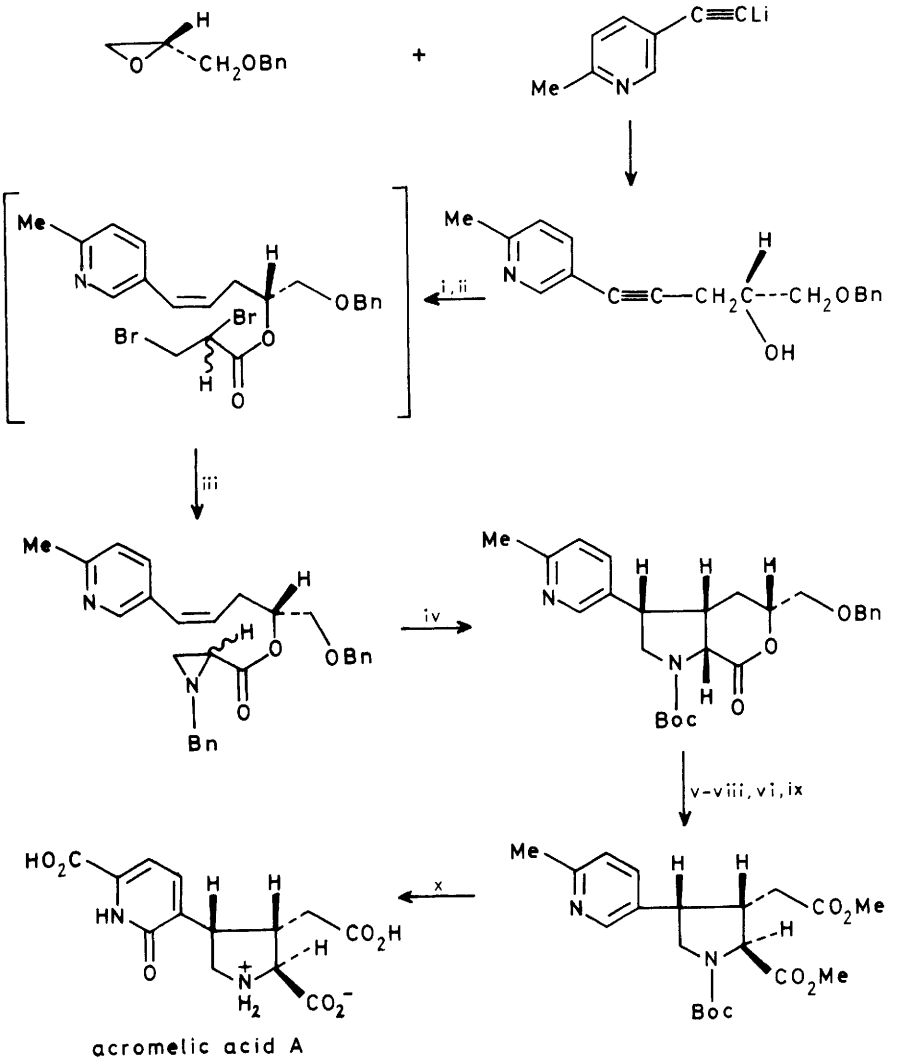
Reagents: i, $PhCH_2OCONO$; ii, [H] and [O]; iii, ZCl, then $Ph_3P/(pyS)_2$; iv, H_2O, H_2/Pd

Scheme 7



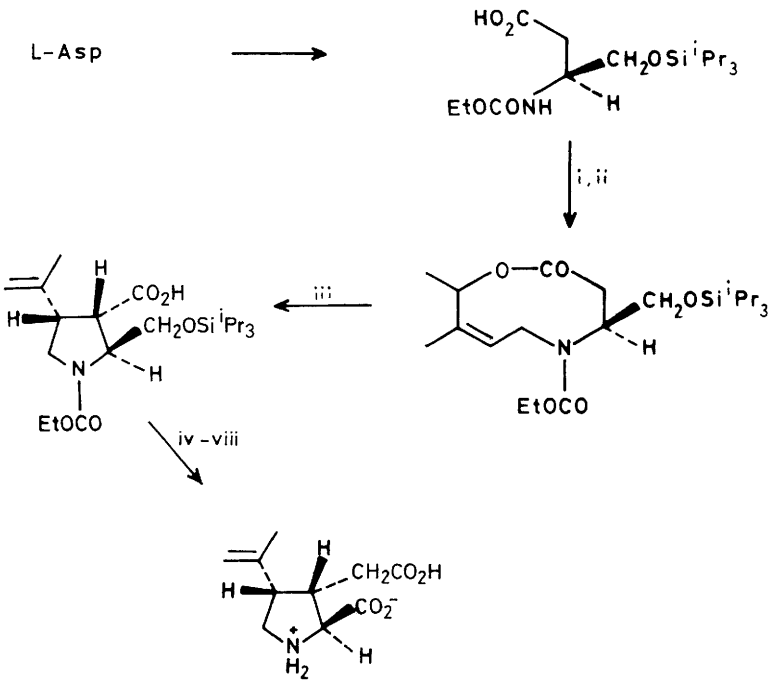
Reagents: i, $Ph_3P{=}CHCO_2Et$; ii, DIBAL/*cis*-ester; iii, MCPBA; iv, NaN_3; v, TosCl; vi, $KOBu^t$; vii, [H]

Scheme 8



Reagents: i, H_2/Lindlar catalyst; ii, $BrCH_2CHBrCOCl$; iii, $PhCH_2NH_2$; iv, 200 °C; v, H_2/Pd—C; vi, $(Boc)_2O$; vii, $NaIO_4$ then $KMnO_4$; viii, Conc. H_2SO_4 (trace), MeOH; ix, NaH; x, established procedures

Scheme 9



Reagents: i, $Pr^i_3SiOCH_2CMe{=}CHCl$ / BuLi; ii, pyridinium toluene-*p*-sulphonate, then 2-chloro-1-methylpyridinium iodide; iii, LDA / Bu^tMe_2SiCl, K_2CO_3; iv, $(COCl)_2$, then CH_2N_2, then $PhCO_2Ag$; v, 40% aq. HF / 20°C; vi, CrO_3 / acetone; vii, Me_3SiI; viii, aq. KOH, then neutralize

Scheme 10

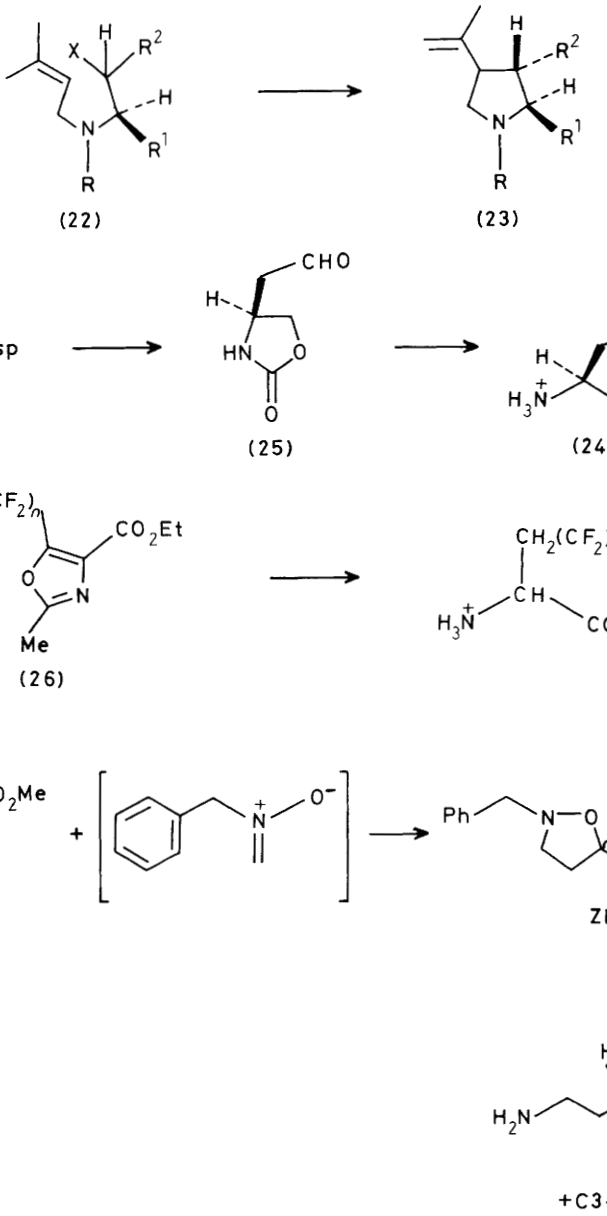
L-amino acids from racemic raw materials. A Volume of Methods in Enzymology is assigned to applications of immobilized aminotransferases in amino acid production.[91] The specific topic of conversion of 5-substituted DL-hydantoins into corresponding L-amino acids using soil bacteria[92] or into D-amino acids using Pseudomonas AJ[93] continues to be studied very thoroughly. Papers covering enzyme reactor technology for production of L-phenylalanine from DL-phenyl-lactic acid,[94] conversion of DL-2-oxo-oxazolidine-4-carboxylic acid into L-serine by Pseudomonas testosteronii[95] and of ethyl α-acetamido-acetoacetate into a mixture of N-acetyl-D-threonine and N-acetyl-L-allothreonine ethyl esters using Saccharomyces rouxii,[96] and a proposed α-proton abstraction mechanism for the biosynthesis of L-azetidinecarboxylic acid from S-adenosylmethionine in Actinoplanes ferrogineus[97] are representative of this topic area.

Syntheses of serine and γ-carboxy-L-glutamic acid, by cathodic reduction of methyl (hydroxyimino)malonamate[98] and by RuO_4 oxidation of a protected L-prolinol (Scheme 6),[99] illustrate specific (non-general) routes to protein amino acids. In the wider context of non-protein natural α-amino acids, continuing interest in canavanine [2-amino-4-(guanidinoxy)butyric acid] is represented in quantitative synthesis from cyanamide and copper(II) canaline in the presence of zinc(II) salts.[90]

The more complex natural products located in this Section all happen to be saturated heterocyclic compounds. Analogues of nicotianamine (19) have been synthesized by condensation of (S)-2-ethoxycarbonylazetidine with ethyl (S)-4-oxo-2-(trifluoroacetylamino)butanoate or ethyl 4-oxobutanoate.[91] A synthesis of tabtoxinine β-lactam ((20) in Scheme 7) in which a nitrone cycloaddition is a key step has been published.[92] Continuing with four-membered ring heterocycles, synthesis of (2R,3S)-oxetin (4) from D-glucose via the derived pyranosylaldehyde ((21) in Scheme 8) has been extended to all 3 stereoisomers.[27] α-D-Glucuronolactone serves as starting point for syntheses of (2R,3R,4R)-2,3-dihydroxyproline and (2R,3R,4R,5R)-3,4,5-trihydroxypipecolic acid and its 2S-epimer using routes briefly described in last year's report (Vol.19, p.10).[93] A related synthesis from the same group is described later (ref.131).

Efficient routes to acromelic acids A and B have been described,[94,95] that by Matsumoto's group[94] confirming structural and stereochemical details (see also Vol.19, p.10). An enantioselective route[95] that is more straightforward than Matsumoto's[94] (which requires L-α-kainic acid as starting material) leading to acromelic acid A is shown in Scheme 9.

A new enantiospecific route (Scheme 10) to (-)-α-kainic acid[96] uses a Claisen enolate rearrangement that achieves the correct stereochemistry at all three chiral centres. This depends of course on chirality already established in the compound subjected to this rearrangement, and this is built into the L-aspartic acid used as starting material. In a related approach, optically pure kainoids with the required (2S,3S)-stereochemistry have been made available through



Reagents: i, LiOH; ii, H_2/Pearlman's catalyst [$Pd(OH)_2$/C]

Scheme 11

cyclization of N-alken-2-yl-L-amino acids in which the carboxy group is first elaborated into a haloalkyl group (22)→(23).[97] Chlorocobaloxime(I) formed in situ is used as the cyclization agent in this route.

4.4 α-Alkyl Analogues of Protein Amino Acids.- Some of the standard general methods are appropriate for satisfying the need for potential enzyme inhibitors of this general class. For example, α-substituted serines were prepared for study as irreversible inhibitors of serine hydroxymethyl transferase through the acetamidomalonate route or through acetoxymethylation of methyl 2-(benzylideneamino)but-2-enoate to give α-vinylserine.[98] Further examples of the latter approach are Michael additions to N-alkylidene alanine and its homologues [99] and preparation of (S)-α-alkylaspartic acids by alkylation by ethyl α-bromoacetate of chiral enolates of oxazolidinones formed from Schiff bases and benzoyl chloride.[100] α-Methylphenylalanine is easily prepared from N-benzylidene-alanine methyl ester by C-benzylation.[101] An example of many applications of the Strecker synthesis to an interesting variation of the present purpose uses a ketone (R^1COR^2), KCN, NH_4OH, and H_2S to give α-amino acid thioamides ($NH_2CR^1R^2CSNH_2$).[102]

A related method, formation of α-spirocyclopropyl amino acids by addition of diazomethane to an activated αβ-unsaturated α-amino acid, has been developed further.[103] The nitrogen atom within the ring of 3-dimethylamino-2,2-dimethyl-2H-azirine is readily acylated by a carboxylic acid accompanied by ring-opening to give N-acyl-α-aminoisobutyroyl dimethylamides, a route that in principle can be extended to any αα-disubstituted α-amino acid.[104]

A review has appeared covering syntheses and uses in peptide synthesis of α-amino-isobutyric acid.[105]

4.5 Models for Prebiotic Synthesis of Amino Acids.- Amino acids continue to be discovered in reaction mixtures that will be familiar to readers of this Chapter over the years. There is still scope for novel variations, as illustrated by the formation of amino acids and amines in aqueous aliphatic carboxylic acid solutions into which an argon-nitrogen plasma is passed.[106] The authors note that this actually represents a new type of catalyst-free nitrogen fixation. Atomic carbon generated in the presence of water and ammonia at 77K leads to glycine, sarcosine, α- and β-alanine, aspartic acid, and serine.[107] This is surmised to account for extra-terrestrial generation of amino acids found in meteorites (in the absence of any other explanation).

Laser irradiation of aqueous ammonium acrylate at 266nm generates mainly α- and β-alanine.[108] Less energetic irradiation (xenon lamp) of solutions of ammonium glycollate containing suspended particulate CdS yields glycine and methylamine.[109]

(Imidazol-4-yl)acetaldehyde and the corresponding glycol are generated in solutions of erythrose, formaldehyde, and ammonia, suggesting a likely prebiotic route to histidine via Strecker reactions.[110] The absence of some simple amino

acids is noticeable when surveying all the experiments of these types. This has been discussed in the context of a hypothesis to the effect that the development of the genetic code and the prebiotic generation of amino acids occurred concurrently.[111]

Further reviews of the topic of this Section have appeared.[112]

4.6 Aliphatic α-Amino Acids.- Sections that follow directly after this cover amino acids for which additional interest resides in side-chain functional groups. This Section serves to collect aliphatic amino acids deserving mention but which are not catered for elsewhere.

After numerous conference papers describing its uses, details of the preparation of 2-amino-4,4-dimethylpentanoic acid (neopentylglycine) have appeared.[113]

Diels-Alder addition of cyclopentadiene to N-acyl dehydroalanine esters gives mixtures of stereoisomers of the expected alicyclic amino acids based on bicyclohept[2.2.1]ene.[114]

Conversion of di-N$^{\alpha}$N$^{\varepsilon}$-t-butoxycarbonyl-L-lysine 2,2,2-trichloroethyl ester into di-N$^{\alpha}$N$^{\varepsilon}$-t-butoxycarbonyl-L-homoglutamine can be achieved by RuO_4 oxidation.[115]

Considerable interest will be generated by the finding that incorporation of the acetal (24) as a histidine replacement in biologically active peptides can give useful analogues. It has been synthesized from L-aspartic acid via its semi-aldehyde derivative (25), formed by Swern oxidation of the corresponding 2-amino alkanol,[116] and the same intermediate has been used in a synthesis of the equivalent dithioacetal and extended through routine steps leading to a novel statine analogue (see Section 4.17).[117]

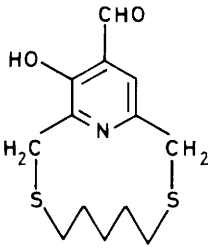
4.7 Alkoxy-α-amino Acids.- Further studies of electrochemical α-methoxylation have concentrated on N-acetyl 4-hydroxyproline esters.[118]

γ-Alkoxy-α-aminonitriles are acceptable substrates for *Brevibacterium* sp.R312 and undergo hydrolysis to the corresponding L-amino acids.[119]

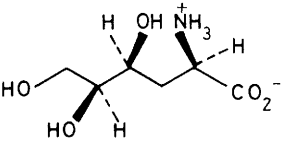
4.8 Halogenoalkyl α-Amino Acids.- β-Fluoroalkyl α-amino acids have been reviewed.[120]

In studies of enzymic amination by 3-methylaspartate ammonia lyase, chloro- and bromofumaric acids were converted into (2R,3S)-3-halogenoaspartates.[121] This implies that enzymic amination of the natural substrate (mesaconic acid) involves re-attack at C2. Amination by ammonia is the final step in a synthesis of DL-hexafluorovaline benzyl ester from benzyl bromoacetate, converted by Ph_3P and hexafluoroacetone into $(CF_3)_2C{=}CHCH_2CO_2CH_2Ph$.[122]

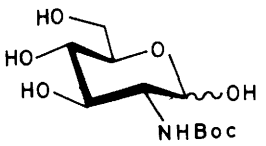
Acylation of ethyl azidoacetate by $CHF_2(CF_2)_nCOCl$ and photocyclization gives the oxazole (26), from which the 2-aminofluoroalkanoic acid is obtained by catalytic hydrogenation and acid hydrolysis.[123]



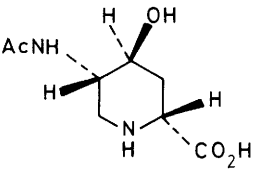
(27)



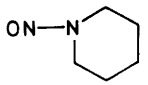
(28)

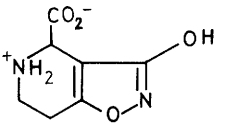


(29)

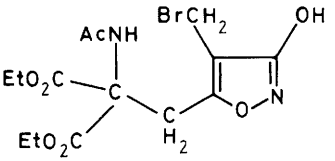


(30)

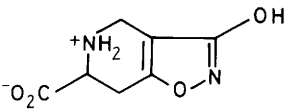




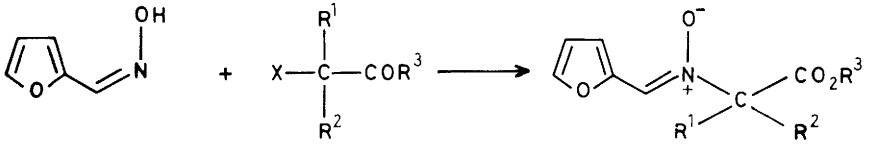
(31)



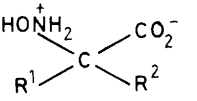
(33)



(32)



(34)



(35)

4.9 Hydroxyalkyl α-Amino Acids.- This class can be divided into acyclic and alicyclic types. Some examples[70-73] have been covered in an earlier section to illustrate asymmetric synthesis.

Electrochemical reduction of L-asparagine[124] and of pyroglutamic acid (pyrrolidone-5-carboxylic acid)[125] yields L-homoserine and (S)-2-amino-5-hydroxypentanoic acid, respectively. A 1.7:1 mixture of allo-L-threonine and L-threonine in 88 and 74% enantiomeric yields, respectively, is obtained through a biomimetic aldol condensation of acetaldehyde with the zinc(II) chelate of the Schiff base of glycine with the pyridoxal-like pyridinophane (27).[126] (3R)- and (3S)-Hydroxy-2S-arginines have been prepared from the corresponding ornithines [these are formed by 1,3-dipolar cycloaddition of the nitrone from $PhCH_2NHOH$ and formaldehyde to methyl N-benzyloxcarbonyl (S)-vinylglycine methyl ester (Scheme 11)].[127]

Established routes to the four ɣ-hydroxyisoleucine diastereoisomers, through photochlorination of L-isoleucine and D-alloisoleucine followed by hydrolysis, have been surveyed.[128] (4,5,6)-Trihydroxynorleucines ((28), and its enantiomer and C-5 epimer) have been prepared[129] from 5,6-O-benzylidene L-ascorbic acid through steps that are, by now, becoming routine and well understood. This is because of an absorption of knowledge and confidence by organic chemists in manipulating simple monosaccharides, illustrated in routes from N-t-butoxycarbonyl-D-glucosamine (29) to (2R,4S,5S)-5-acetamido-4-hydroxypipecolic acid (30),[130] and from D-glucuronolactone via amination of (4S,5S)-5,6-dihydroxyhex-2-en-4-olide en route to (2S,4S,5S)-dihydroxypipecolic acid and bulgecine (see also refs. 93, 337).[131]

Enzymic transamination (immobilized glutamic oxaloacetic aminotransferase) effects the conversion of ɣ-hydroxy-α-ketoglutaric acid to ɣ-hydroxy-L-glutamic acid. An interesting explanation for the success of this otherwise sluggish conversion lies in the choice of the amination agent, cysteinesulphinic acid, which generates a driving force for the reaction through the instability of the corresponding keto-acid that readily breaks down into SO_2 and pyruvic acid.[132]

4.10 α-Amino Acids with Unsaturated Side Chains.- α-Azidocinnamates, formed by condensation of ethyl azidoacetate with aromatic aldehydes, yield N-carboxy anhydrides of dehydro-phenylalanine and tyrosine through reaction with phosgene.[133] This general process has been extended[134] to ornithine and lysine analogues through Wittig-type condensation of N-benzyloxycarbonylamino aldehydes with methyl N-benzyloxycarbonyl α-diethoxyphosphinylglycinate in the presence of ^{t}BuOK.

L-Vinylglycine has a number of practicable syntheses on offer, supplemented now by elimination from the L-methionine-derived Seebach oxazolidinone (cf. ref 70).[135] 3-Chlorovinylglycine has been prepared through chlorination of N-benzyloxycarbonyl vinylglycine methyl ester,[136] though the same workers choose to

prepare the 3-fluoro analogue through Strecker synthesis starting with 2-fluoroacrolein.[136] These turn out to be some 800 times more effective as irreversible inhibitors of alanine racemase from E.coli, compared with β-fluoro-substituted alanines. Since β-fluoro-D-alanine is a potent broad-spectrum orally active antibiotic, much pharmaceutical promise is offered by these halovinylglycines.

Allyl selenides in the presence of amines undergo oxidative [2,3]-sigmatropic rearrangement accompanied by nucleophilic addition, and this offers an entry to D-allylglycines starting from αβ-unsaturated alkanoates.[137]

Similar methods have led to the synthesis of (E)-3,4-dehydroglutamic acid, in a continuing search for potential Vitamin K-dependent carboxylase inhibitors.[138] γ-Methylene-L-glutamic acid and the (E)-ethylidene analogue have been prepared through ring-opening of ethyl R-[N-(p-nitrobenzoyl)]aziridinecarboxylate with $Ph_3P{=}CHCO_2Et$ (to give an isolable ylide), followed by Wittig reaction with formaldehyde or acetaldehyde, respectively.[139]

4.11 α-Amino Acids with Aromatic and Heteroaromatic Side-chain Groups.- There are many examples in the later Section 6.3, describing specific reactions of amino acids (i.e. reactions of side-chain functional groups), which amount to the conversion of one of the familiar aromatic or heterocyclic amino acids into others of the same class.

DL-o-Carboxyphenylglycine (noticed to be a potential glutamic acid substitute) has been synthesized from phthalonic acid ($o\text{-}HO_2C.C_6H_4.CO.CO_2H$) and NH_3 followed by $NaBH_4$ reduction of the resulting Schiff base.[140] Two well explored enantioselective routes have been explored for the synthesis of homotyrosines, one involving conventional azlactone synthesis followed by asymmetric hydrogenation [H_2/chiral Rh(I) - phosphine catalyst] and the other involving Friedel-Crafts acylation of chloroanisoles with (R)-aspartic anhydride.[141]

β-(N-Indolyl)-L-alanine has been isolated from fermentation of L-serine with indoline and E.coli.[142]

Syntheses of ibotenic acid analogues (31), through construction of the isoxazole ring on the pipecolic acid moiety, and (32), through cyclization of the substituted acetamidomalonate (33), have been reported.[143]

Regiospecific alkylation of N^{τ}-N^{α}-di-t-butoxycarbonyl-L-histidine by alkyl triflates or mesylates yields N^{τ}-alkyl-L-histidines.[144]

4.12 N-Substituted α-Amino Acids.- This Section excludes N-protected amino acids (representative coverage of these will be found in Section 6.2) but deals with N-hydroxamino acids, hydrazino acids, and their close relatives that are of interest, inter alia, in the synthesis of modified peptides.

N-Alkylation of furan-2-aldoxime by an α-halogeno-ester, followed either by acid hydrolysis or by reaction with hydroxylamine, yields an α-N-hydroxamino acid ((34)→(35)).[145] Alkylation of O-benzyl- or O-trityloximes of α-keto acids using

an alkyl-lithium gives the corresponding hydroxyamino acid.[146] These compounds may also be obtained from α-hydroxy esters by treatment with diethyl azodicarboxylate and Ph_3P in the presence of N-trichloroethoxycarbonyl- or N-benzyloxycarbonyl-O-benzylhydroxylamine [147] or by conversion into triflate esters and S_N2 displacement by O-benzylhydroxylamine.[148] HCN reacts with nitrones derived from secondary amines by oxidation with SeO_2 or by H_2O_2 and Na_2WO_4 and leads to α-cyano-hydroxylamines, from which the corresponding hydroxy-imino acid is readily obtained by hydrolysis of the cyanide group.[149] A similar side-chain N-hydroxylation procedure with N^{α}-benzyloxycarbonyl-N^{ε}-acetyl lysine t-butyl ester, using benzoyl peroxide, has been described.[150]

Hydrazino acids have been prepared by Shestakov rearrangement of hydantoic acids $H_2NCONHCHRCO_2H$ through treatment with KOCl.[151] Conversion of norbornyl esters into silyl enolates followed by treatment with di-t-butyl azodicarboxylate and $TiCl_4$ gives (S)-bis(Boc)hydrazino esters of high enantiomeric purity with efficient recovery of the norbornyl alcohol auxiliary.[152]

Guanidino acids may be prepared in good yields by reaction of amino acids with guanidine C-sulphonic acids $R'N{=}C(NHR^2)SO_3H$ (obtained from the corresponding thiourea and performic acid).[153]

Betaines are made easily from simple amino acids by alkylation, but an N^{α}-protection strategy is needed for lysine for the synthesis of the N^{ε}-trimethyl betaine analogue.[154]

4.13 α-Amino Acids containing Sulphur or Selenium.- Michael addition of thiols to α-cyano-ethenyl esters, followed by amination with NH_3, leads to S-alkyl-cysteines.[155] Similar application of established methods has led to series of α-monosubstituted and ββ-disubstituted cysteines.[156]

An improved synthesis of S-adenosylmethionine involves reaction of the sodium salt of homocysteine thiolate with 5'-chloro-5'-deoxyadenosine followed by S-methylation using trimethylsulphonium iodide.[157]

N-Acetylselenocysteine N-methylamide has been prepared by standard methodology.[158] New approaches to the synthesis of L-selenocystine and L-selenomethionine involve the carbon radical approach in which N-Boc-L-glutamic and aspartic α-benzyl esters are converted into N-hydroxypyridine-2-thione esters, and these are irradiated in the presence of dimethyl diselenide.[159]

4.14 Phosphorus-containing α-Amino Acids.- Analogues of the amino acids in which the carboxy group is replaced by a phosphorus oxyacid grouping are excluded.

Mention has been made earlier of amino acids containing side-chain phosphorus functional groups,[53] and a further example that is representative of straightforward synthetic approaches is found in the reaction of oximinophosphonate esters: $(EtO)_2P(O)C(CO_2Et){=}NOCOR$ yield insertion products with diazomethane which on Al/Hg reduction and hydrolysis give β-phosphonylalanine.[160]

4.15 α-Amino Acids Synthesized for the First Time.- Inclusion of this Section should not be taken to suggest that this Chapter offers a complete listing of all new α-amino acids in the period under review. Its purpose is to net examples that have not found a place in other parts, and its presence helps to avoid further proliferation of the Chapter into small sub-sections. The Journal of Medicinal Chemistry is well known as a location for compact presentation of research leading to synthesis of large numbers of closely related analogues, and this information is not comprehensively repeated in this Chapter.

2-Amino-3-boronopropionic acid, an analogue of aspartic acid in which the side-chain function is replaced by a boronic acid grouping, has been prepared by acetamidomalonate and Curtius routes.[161] 3'-Deoxy-modified S-adenosyl-L-homocysteines have been described.[162]

4.16 Labelled Amino Acids.- All examples are based on familiar protein amino acids and are cited in order of increasing atomic number of the labelling isotope. Most attention has been given to introduction of hydrogen and carbon labels, of course, and the special demands for rapid synthesis and purification imposed by the short half-life of ^{11}C offer fascinating and more widely useful insights into the exploitation of the organic chemist's skills.

The simplest labelling of the simplest α-amino acid is represented in reduction by Saccharomyces cerevisiae of [2 - ^{2}H]furfural to provide a substrate for production of (R)-[2 - ^{2}H]glycine.[163] (R)-[2 - ^{2}H]serine (from which the corresponding β-fluoroalanine was prepared by SF_4/HF) has been synthesized by ^{2}H exchange.[164] This was achieved by conversion of DL-serine isopropyl ester into 2-phenyl 4-isopropyloxycarbonyl oxazoline using methyl benzimidate and deprotonation with Ph_3CLi followed by quenching with $AcO^{2}H$, and classical resolution of the labelled serine as its (-)-bromocamphorsulphonate. Doubly ^{2}H-labelled L-phenylalanine (the α-proton and the ring o-proton) has been prepared from E-[2,2' - ^{2}H]-cinnamic acid through standard manipulations.[165]

Tritium labelling has been accomplished using $^{3}H_2$ to give L-[β,γ - ^{3}H]lysine, starting from the side-chain chlorination product of L-lysine.[166] More controlled approaches employing $^{3}H_2$ - Pd/C are embodied in syntheses of [3,4,5,5 - $^{3}H_4$]-L-ornithine (from which the correspondingly labelled L-arginine was obtained using NH_2CN and MeSH) and of [3,4 - $^{3}H_2$]-DL-glutamic acid, using β-(β-cyanovinyl) acetamidomalonate.[167] Similar approaches from β-(4-chlorobut-2-ynyl) acetamidomalonate lead to [4,5,6,6 - $^{3}H_4$]-L-lysine, [4,5,5,5 - $^{3}H_4$]-L-leucine, and [2,2,3,3 - $^{3}H_4$]-α-aminoisobutyric acid.[168]

The 1- and 3-[^{11}C]-labelling studies described in a surprisingly large number of papers this year illustrate several different standard methods of α-amino acid synthesis. [1 - ^{11}C]Glycine, prepared within 30-35 minutes by the Bucherer-Strecker synthesis from $^{11}CN^-$, formaldehyde and ammonium carbonate,[169] represents

a slow rate of production compared with that for D- and L-[1 - ^{11}C]tyrosines, prepared by the same route within 20 minutes.[170] Further Strecker reaction studies using H^{11}CN,[171] including their acceleration with an apparatus permitting operations at high temperatures combined with high pressures,[172] have been described. Carboxylation of α-lithioisocyanides with $^{11}CO_2$ offers an alternative route to [1 - ^{11}C]amino acids and has been exemplified with a synthesis of DL-[1 - ^{11}C]methionine.[173]

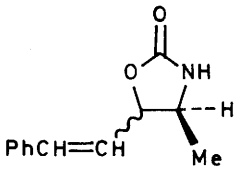
[3 - ^{11}C]-L-Alanine has been prepared by asymmetric alkylation with $^{11}CH_3I$ of [(+)-2-hydroxypinan-3-ylidene]glycine t-butyl ester, deprotonated with 2,2,6,6-tetramethylpiperidyl-lithium.[174] A total of 85 minutes is required for the synthesis of [3 - ^{11}C]-L-valine, using phase-transfer alkylation of $(Ph_2CH)_2NCH_2CO_2Bu^t$ by $Me_2{}^{11}CHMgI$ and resolution using D-amino acid oxidase (35 minutes).[175]

[3 - ^{11}C]-DL-Phenylalanine has been prepared from the azlactone of [1 - ^{11}C]benzaldehyde, not by the obvious hydrogenation and hydrolysis route but via the less well-known alkaline hydrolysis to the labelled phenylpyruvic acid, followed by amination.[176] The same role is given to an azlactone in a synthesis of [^{15}N, 2 - ^{13}C]-L-tyrosine from [2 - ^{13}C]glycine; the α-keto-acid is aminated to L-tyrosine using aspartate transaminase.[177] Detailed study of biosynthetic pathways is also the purpose of synthesizing [^{15}N, ^{13}C]-L-glutamic acid by Brevibacterium flavum using differently labelled acetate to provide appropriate patterns of ^{13}C-labelling.[178] [2' - ^{13}C]-DL-Tryptophan has been prepared by alkylation of formamidomalonate with the Mannich reaction product of [2 - ^{13}C]indole, dimethylamine, and formaldehyde.[179] [^{15}N, 2 - ^{13}C]-L-Phenylalanine[165] and [1 - ^{13}C]-DL-1-amino-4-carboxycyclohexane-1-carboxylic acid[180] have been prepared also by applications of standard amino acid syntheses.

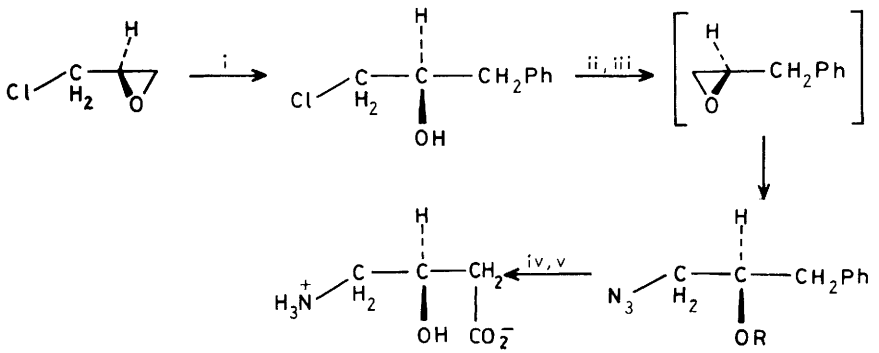
Conventional routes have been published for [3 - ^{14}C]-3-(2-naphthyl)-D-alanine [asymmetric hydrogenation of $AcNHC(=^{14}CH\text{-}C_{10}H_7)CO_2R$],[181] [1 - ^{14}C]-L-phenylalanine [Strecker synthesis and enzymic resolution (thermolysin)],[182] and [methyl - ^{14}C]-L-methionine ($^{14}CO_2$ to $^{14}CH_3I$ by $LiAlH_4$ and HI, and methylation of protected L-homocysteine).[183]

As mentioned above, introduction of nitrogen isotopes in the synthesis of labelled amino acids has the special option of enzymic amination available,[78,132] and syntheses of ^{13}N-γ-aminobutyric acid from α-ketoglutaric acid using immobilized glutamate decarboxylase[184] and of ^{15}N-L-alanine from lactic acid, $^{15}NH_4Cl$, NADH, and immobilized L-alanine dehydrogenase[185] are further examples. Synthesis of (π-^{15}N)- and (π,τ-$^{15}N_2$)-labelled histidines involves construction of the imidazole ring on to appropriately labelled 2,5-diamino-4-oxopentanoic acids by standard methods.[186]

To the large number of papers already published on ^{18}F-labelled 6-fluorodopa [6-fluoro-(3,4-dihydroxyphenyl)-L-alanine] are added two more,[187,188] both using [^{18}F]acetyl hypofluorite for electrophilic substitution of protected dopas. In

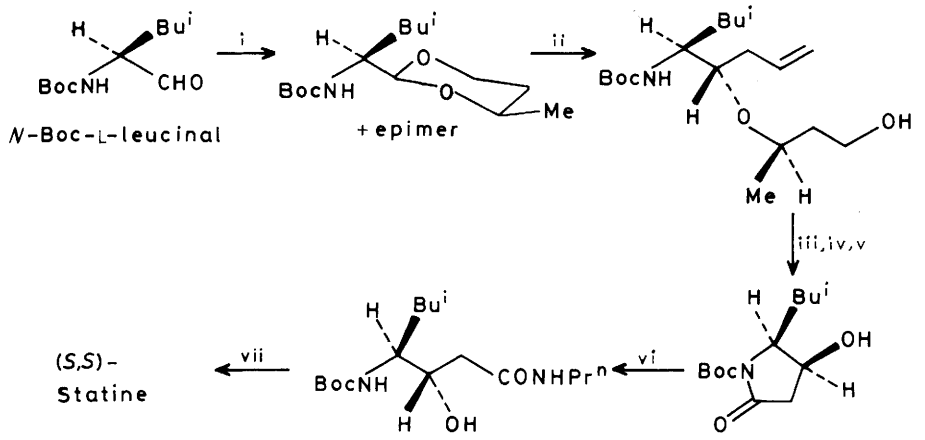


(36)



Reagents: i, PhLi; ii, NaN_3; iii, Boc_2O; iv, Sharpless oxidation, then TFA; v, H_2/Pt

Scheme 12



Reagents: i, (*R*)-1,3-dihydroxypropane, TsOH, CH_2Cl_2/reflux; ii, TMS-$CH_2CH{=}CH_2/TiCl_4$; iii, O_3; iv, pyridinium chlorochromate; v, $(PhCH_2)_2\overset{+}{N}H_2$ $CF_3CO_2^-$; vi, Pr^nNH_2; vii, deprotection

Scheme 13

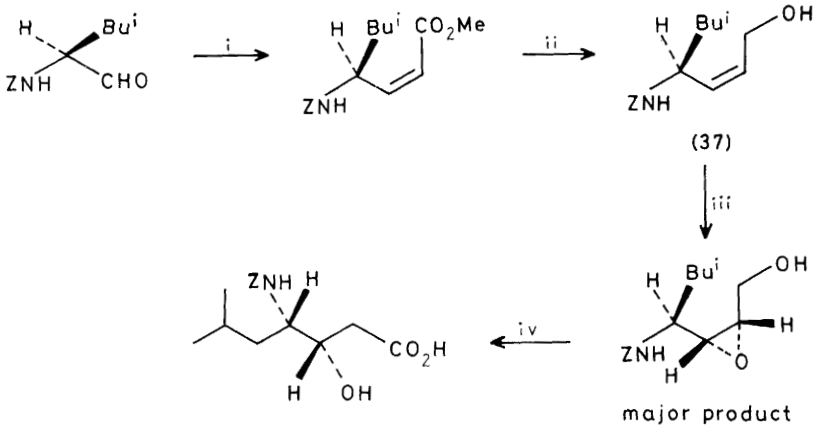
one of these, particular attention was paid to achieving synthesis and h.p.l.c. purification in the minimum time so as to achieve maximum radiochemical yield, though this was only 8% at the end of 100 minutes' work.[188]

Syntheses of [^{35}S]-adenosylhomocysteine[189] and [^{75}Se]-methionine[190,191] employ simple reagents for introduction of the labels (demethylation of commercially available [^{35}S]-L-methionine for the synthesis of homocysteine, followed by alkylation;[189] $Me^{75}SeNa$[190] and $Me^{75}SeLi$[191] for reaction with L-2-amino-4-bromobutanoic acid).

4.17 Synthesis of β-Amino Acids and Higher Homologous Amino Acids.- Hydroxylated β-amino acids that were discovered as unusual components of peptide antibiotics have started to dominate this Section, which is usually occupied by less spectacular ω-amino acids, such as γ-aminolaevulinic acid - shown to be formed from glutamic acid in plant chloroplasts.[192] Studies of glutamic acid α-semi-aldehyde as a potential intermediate in the C-5 tetrapyrrole biosynthetic pathway have been published in a paper that also describes surprising failures in applications of standard synthetic manipulations in exploration of routes to this γ-amino acid.[193]

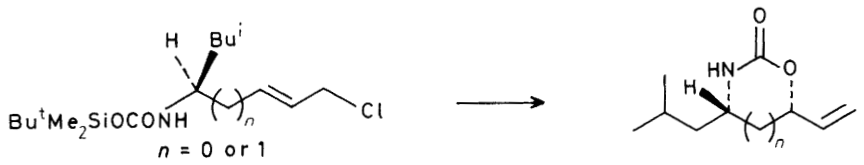
Asymmetric syntheses of intrinsic interest have been published for other close relatives of protein amino acids. These apply to several hydroxylated ω-amino acids, including (R)-isoserine, synthesized in good yield from di-azidomannitol through conventional stages in a route that is readily adaptable to lead to the (S)-enantiomer.[194] "D-isothreonine" and "L-allo-isothreonine" [(2R,3S)- and (2S,3S)-3-amino-2-hydroxybutanoic acids, respectively] have been prepared by $RuCl_3/NaIO_4$ oxidation of the styryloxazolidin-2-one (36) obtained from L-alanine.[195] The homologue, γ-amino-β-hydroxybutanoic acid ("GABOB"), of interest as a GABA analogue, has been prepared from (R)-epichlorhydrin in 57% overall yield through six steps (Scheme 12), representing a distinct improvement over existing methods.[196] Perhaps most obvious of all as a route to (S)-2- or -4-amino-5-hydroxypentanoic acids, the selective reduction of L-glutamic acid derivatives, has been achieved by $LiAlH_4$ reduction of N-trityl α- or γ-methyl esters.[197] At the other end of the scale, sophisticated strategies are being developed for synthesis of statine and, particularly, "MeBmT" [2S,3R,4R,6E-3-hydroxy-4-methyl-2-(methylamino)-6-octenoic acid].

Statine [(3S,4R)-3-hydroxy-4-amino-6-methylheptanoic acid] is approachable by chain extension of the carboxy group of an α-amino acid, and Boc-D-leucine offers a suitable starting point.[198] Conversion into the acylimidazole and nucleophilic substitution with $LiCH_2CO_2R$ gives the β-keto-ester $^tBuCH(NHBoc)COCH_2CO_2R$, which gives a mixture of statine and 3R-epimer on hydride reduction and deprotection. The same chain extension technique is used in the synthesis of a statine analogue in which the two methyl groups of the isobutyl group are replaced by sulphur atoms as part of a dithiolane moiety.[117] Here, N-benzyloxycarbonyl-L-aspartic acid is



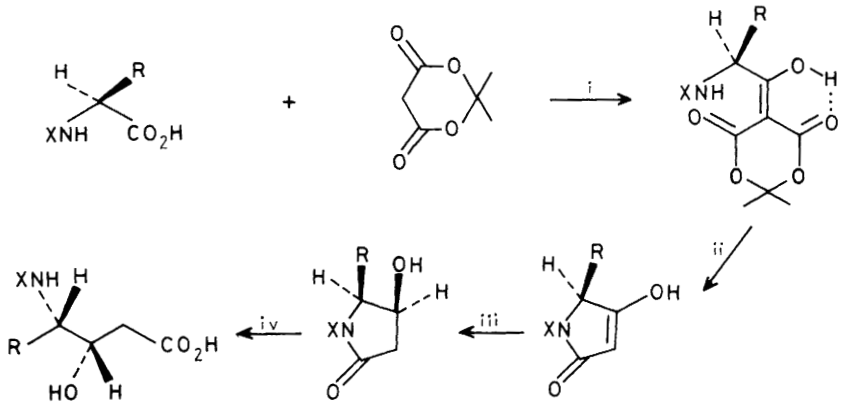
Reagents: i, $(CF_3CH_2O)_2P(O)CH_2CO_2Me$; ii, DIBAL; iii, MCPBA; iv, O_2/Pt

Scheme 14



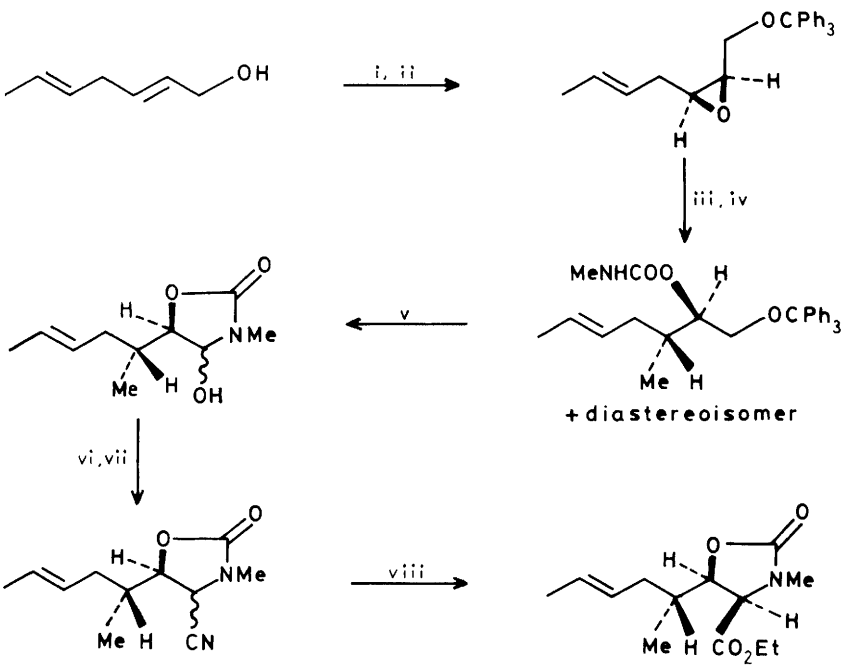
Reagents: i, AgF-Pd^{II}

Scheme 15



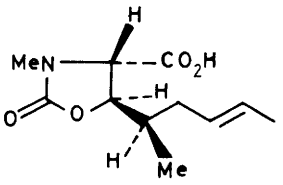
Reagents: i, isopropenyl chloroformate, DMAP; ii, reflux in MeCN or EtOAc, 20 min; iii, $NaBH_4$; iv, 1M NaOH

Scheme 16

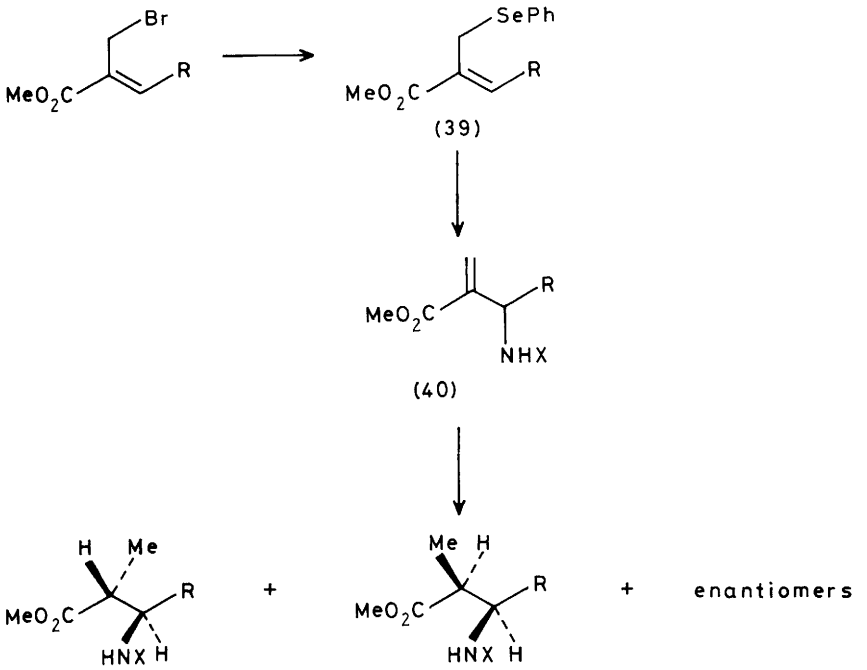


Reagents: i, Bu^tOOH; ii, Ph_3CCl; iii, Me_2CuLi, $BF_3 \cdot Et_2O$; iv, MeNCO; v, $(COCl)_2$; vi, Ac_2O; vii, Me_3SiCN; viii, K_2CO_3 then EtOH

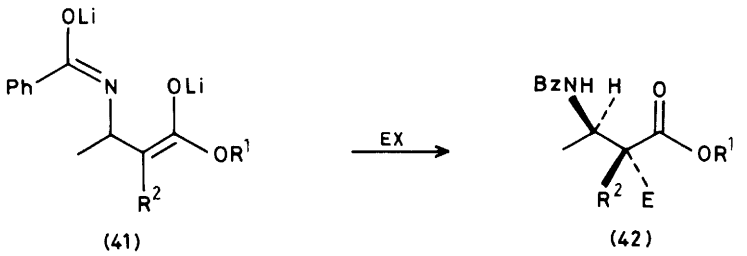
Scheme 17



(38)


Br
MeO2C
R
SePh
(39)
NHX
(40)
H
Me
HNX
+
enantiomers

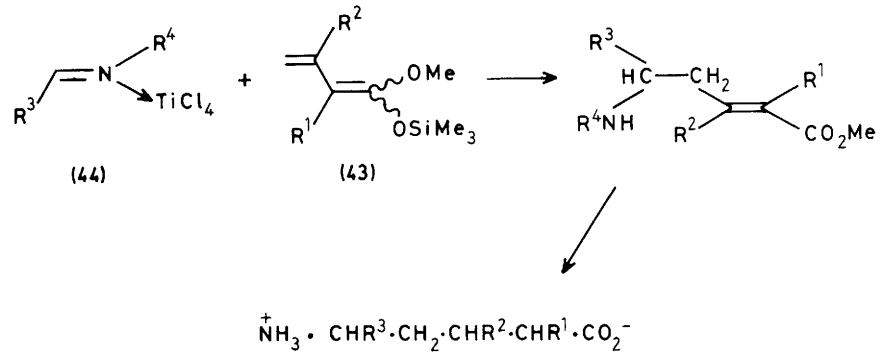
Scheme 18


OLi
Ph
N
OR1
R2
(41)
EX
BzNH
H
O
E
(42)

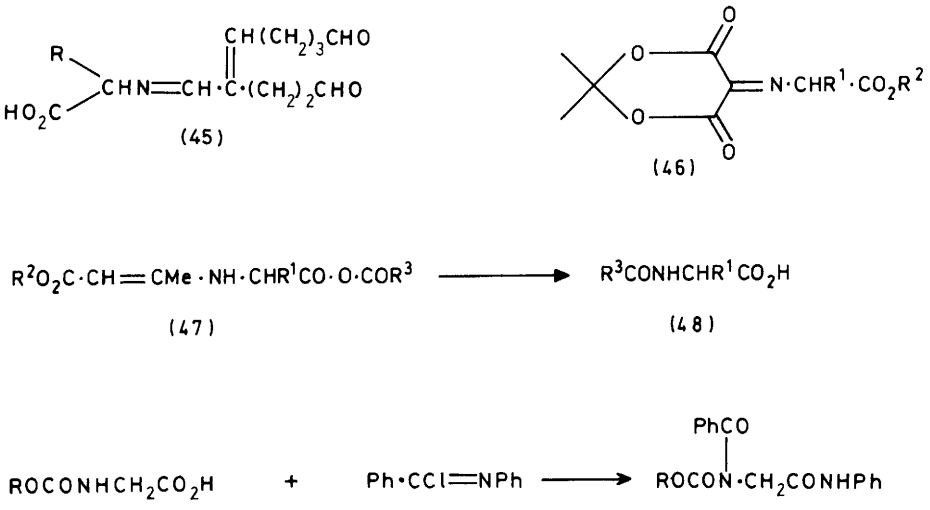
the starting material, the derived chiral oxazolidinone (25) being opened with $LiCH_2CO_2R$. The other routes to statines in this year's literature employ nucleophilic attack on an N-Boc-γ-lactam (Scheme 13),[199] diastereoselective epoxidation of the appropriate cis-4-(benzyloxycarbonylamino)allyl alcohol ((37) in Scheme 14),[200] and a related use of an allyl chloride (Scheme 15).[201] The last-mentioned route explores the usefulness of the silylcarbamate group in stereoselective access to 1,2- and 1,3-amino-hydroxyl systems, both in the statine case and also for the synthesis of (3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid. Activation of an N-protected amino acid through mixed-anhydride formation with isopropenyl chloroformate has been used[202] to prepare a suitable derivative for coupling to Meldrum's acid. Of several bases tried, only 4-dimethylaminopyridine was a suitable catalyst for this process, the start of a route to N-Boc-statines via N-protected tetramic acids (Scheme 16).[202]

The problems of synthesis of "MeBmT", the cyclosporin constituent, seem much less formidable as a result of several recent reports. Earlier routes involved up to 24 separate steps; this has been cut back considerably for new routes by Rich and co-workers, in one of which[203] the lithium enolate of N-(p-methoxybenzyl)-sarcosine is added to (2R,4E)-$CH_3CH{=}CHCH_2CHMeCHO$ to give (38) and its diastereoisomer after ethanolic KOH treatment. Resolution of (38) with (+)-ephedrine followed by hydrolysis gives MeBmT. Routes by Evans[204] and by Schmidt[205] use the same 2-methylhexanal as starting material; the latter authors add to it the lithium enolate of N,N-bis(trimethylsilyl)glycine trimethylsilyl ester. As an alternative to Rich's route just described[203] (which the authors have christened a 'short synthesis'), a route starting with allylic epoxidation of hepta-2,5-dienol has been reported by Rich's group (Scheme 17).[206]

More generally applicable methods for the synthesis of β-amino acids continue to be developed. Tin(II) carboxylic thiolester enolates formed *in situ* from tin(II) 2-methyl-2-propanethiolate and ketenes react stereoselectively (anti-addition) with imines in the presence of tin(II) triflate to give β-amino acid thiolesters.[207] Amination of allyl selenides gives protected β-amino-α-methylene-alkanoic acid esters ((39)→(40) in Scheme 18); homogeneous-catalyzed hydrogenation of these esters is strongly diastereoselective, depending on the nature of the N-substituent.[208] More routine results arise from the modification of the α-carboxy function of N-formyl dibenzyl aspartate to give β-amino-γ-ketobutyric acid derivatives, including Dakin-West manipulation of 4-benzyloxycarbonylmethyloxazolin-5-one derived from this aspartic acid derivative.[209] The other obvious way to build up αβ-disubstitution patterns on a β-amino acid is by alkylation, and methyl or ethyl N-benzoyl-3-aminobutanoates can be stereoselectively alkylated after dilithiation with LDA.[210] The incoming group is directed to create products of l- and u,u - configuration, through alkylation with lk-1,2-induction (to use Seebach's terminology[210]), i.e. the electrophile approaches the enolate β-carbon atom from the least-hindered direction ((41)→(42)).



Scheme 19



γ-Amino acids may be obtained from corresponding keto-esters by Meerwein-Ponndorf-Verley reduction, substitution of OH by Cl with $SOCl_2$, and amination (NH_3).[211] A more sophisticated approach leading to δ-amino acids is based on the reaction of vinylketene silylacetals (43) with imine - $TiCl_4$ complexes ((44) in Scheme 19).[212]

4.18 Resolution of Amino Acids.- Examples of classical methods based on diastereoisomeric salt formation of a DL-amino acid or an N-substituted derivative with a chiral amine or acid are embedded in some of the papers located in other sections of this Chapter. A further example of this type, with the added interest of an accompaniment of asymmetric transformation (DL-p-hydroxyphenylglycine is converted into its D-enantiomer to the extent of 80%), employs (+)-phenylethanesulphonic acid as chiral reagent.[213] Equilibration of N-(2-naphthyl)-D-alanine 1-undec-10-enyl ester with triethylamine in the presence of the stereolabile 2,4-dinitrobenzoyl-DL-leucine n-butylthiolester gives 80% enantiomeric excess of the D-leucine isomer. This interesting example of asymmetric induction points to a stabilizing enantioselective association between the respective D-enantiomers.[214]

The preferential-crystallization technique depends, perhaps arbitrarily, upon the intimate details of crystal habit. Quaternary ammonium salts of N-formyl-DL-amino acids, for example, have been found to crystallize in the essential characteristic form (each crystal composed exclusively of one enantiomer) for preferential crystallization. Among several further examples in this year's literature, 1,1,3,3-tetramethylbutylammonium salts of N-formyl-DL-α-phenylglycine,[215] DL-tyrosine,[216] or DL-phenylalanine,[217] ammonium N-acetyl-DL-norleucinate,[217] and DL-thiazolidine-4-carboxylic acid[218] have been resolved by this technique.

Column chromatographic methods are simple and often very effective, for example for resolution of DL-amino acids over cellulose.[220] Discriminatory ratios have been calculated to be close to 1% for adsorption of D-alanine relative to its L-enantiomer on crystalline cellulose,[221] though it must be said that practitioners would establish characteristics such as these on an empirical basis when upscaling their laboratory experiments. Optically active cationic complexes, such as copper(II)-L-lysine, adsorbed on clays (montmorillonites) show modest selectivity coefficients for DL-amino acids, the L-enantiomer being more strongly adsorbed from solutions at neutral pH.[222] There are numerous papers on the h.p.l.c. variant of this approach, using a variety of chiral phases, and those on analytical resolutions are described in Section 7.4.

The demands of clean separation on the analytical scale have led to the development of new chiral stationary phases that are suitable for preparative resolution. Silica linked to (R,R)-tartramide through undecamethylene spacer chains provides a substantial discriminatory ratio between D- and L-amino acids

based on hydrogen-bonding interactions,[223] and an (R,R)-tartaric acid mono-n-octylamide-copper(II) modified stationary phase operating on the ligand exchange mechanism for resolution of DL-amino acids has been described.[224] The Pirkle-Pochapsky chiral stationary phases (CSPs) based on N-(3,5-dinitrobenzoyl)-L-amino acids[225] continue to be studied, showing substantial chiral recognition for enantiomers of N-aryl-α-amino acid esters[226,226] with discriminatory ratios larger than 18:1 at room temperature for 10-undecenyl esters of N-2-(naphthyl)amino acids.[226] A new chiral stationary phase, formed by attaching [N-(S)-(1-α-naphthylethyl)amino-carbonyl]-L-valine to γ-aminopropylsilanized silica, shows promise for chromatographic resolutions of amino acids.[227]

Tailor-made polymers, represented by acrylamide - divinylbenzene - ε-itaconyl-L-lysine copolymer and by methacrylic acid - ε-methacryloyl-L-lysine NN'-methylene bis(acrylamide) copolymer, have been used for resolution of DL-lysine; the former copolymer permits emergence of the D- before the L-isomer, and the reverse sequence is seen with the latter copolymer for the same amino acid.[228] A simple approach in which silica is coated with proteins in this way is successful for the resolution of N-benzoyl- or -dansyl-amino acids,[229] and a related approach,[230] based on zwitterion pairing of a DL-amino acid with a di- or tripeptide in solution, has been studied using DL-tryptophan.[230]

Diastereoisomeric salt or complex formation, as in the last-mentioned example, is represented in chromatographic separation of a chiral crown ether - DL-amino acid pair, using aqueous perchloric acid as mobile phase.[231] The same principle is exploited in ligand exchange resolution of DL-amino acids using (1R,2S)- or (1S,2S)-2-carboxymethylamino-1,2-diphenylethanol as chiral additive.[232] More examples are discussed in the context of h.p.l.c. analysis in the later Section 7.4 of this Chapter.

Enzymic methods are represented (see also ref.44) in a use of α-chymotrypsin for the stereoselective hydrolysis of N-acetyl erythro-β-(p-nitrophenyl)serine esters to give the (2S,3S)-amino acid.[233] N-Acetyl-D-amino acids accumulate through the action of fermenting yeast proteinases on corresponding DL-esters.[234] All four enantiomers of δ-hydroxylysine have been secured through mould acylase-catalyzed hydrolysis of N^{α}-formyl-N^{ϵ}-benzyloxycarbonyl-DL-lysine esters and their allo isomers.[235] Applications of enzymes in the production of protein L-amino acids have been reviewed in the earlier Section 4.3, the principle being applicable for the synthesis of unusual L-amino acids (e.g. from keto-acids using aspartic aminotransferase[70]).

No year passes without a few references in the literature to experiments (and many references to speculations) on the predominance in the present biosphere of the L-enantiomers of amino acids. Reviews have appeared on this topic, a general coverage,[236] and specific attention to parity violation in chemical reactions, with special reference to the formation of α-aminonitriles ($CN^- + CH_3CH{=}N^+H_2$).[237] In the latter context, the combination of differential degradation by chiral β-

radiation with the weak parity violation inherent in an individual enantiomer[238] is concluded to require a very long time-scale (1500 y) before any noticeable selection could occur.[239]

It has been surmised that prebiotic conditions could not have been favourable for the enantiospecific role given to chiral β-radiation in the enantioselective degradation of racemates.[240] An experiment that will undoubtedly be the subject of further scrutiny, like all others of its type that make fundamental claims, has shown that chiral positrons from ^{22}Na decay interact differently with the enantiomers of alanine.[241]

Frank's model[242] has been given further theoretical support.[243] The model assumes local deviations from equilibrium in reactions of the separate enantiomers of a racemate with large excesses of achiral compounds and the merging of these localities so as to tend to "extinguish" one enantiomer over long periods of time. Weak neutral currents influence rate constants differently for reactions of each enantiomer and determine that those localities rich in one enantiomer will tend to accumulate more rapidly than localities rich in the other enantiomer.

5 Physical and Stereochemical Studies of Amino Acids

5.1 Crystal Structures.- Amino acids subjected to X-ray crystal structure determination, as reported in the recent literature, are β-alanine,[244] L-citrulline,[245] and L-methionine sulphoximine [the "natural" isomer, shown to possess the (S,S)-configuration[246]].

A substantial crop of simple amino acid derivatives is represented in the other crystal structure papers this year, covering L-phenylalanine benzyl ester hydrochloride,[247] S-benzyl-L-cysteine methyl ester hydrochloride,[248] N-(β-phenylpropionyl)glycine ethyl dithioester,[249] N-phenylacetyl-L-phenylalanine,[250] N^{α}-acetyl-L-arginine ethylamide perchlorate,[251] N-acetyl-N-hydroxy-DL-alanine,[252] N-trityl-L-aspartic acid dibenzyl ester and N-trityl-L-leucine benzyl ester,[253] N-benzyloxycarbonyl-γ-carboxy-L-glutamic acid γγ-di-t-butyl ester α-methyl ester,[254] and N-acetyl-2,4-methanoproline methylamide.[255] Interest in the topic of the last-mentioned paper lies in the fact that the formally achiral molecule adopts mirror-image enantiomeric forms in the crystal, due to distortions accompanying packing.

5.2 Nuclear Magnetic Resonance Spectrometry.- Attention continues to spread to other nuclei - i.e. beyond ^{1}H and ^{13}C - but novel and relevant applications continue to be explored for all n.m.r.-active nuclei.

Configurational assignments may be made to α-amino acids and their esters on the basis of consistent shift differences of the α-proton resonance for each enantiomer in the presence of either of the chiral shift reagents (R)-propylenediaminetetra-acetato europium(III)[256] or Eu(d-facam)$_3$.[257] D- and L-

enantiomers of 22 amino acids were studied in aqueous solutions containing the former of these reagents.[256] In all cases, the Hα-resonance showed larger upfield shifts for an L-enantiomer than for its D-isomer. The method is not suitable for estimation of enantiomer ratios for amino acids since accurate integration of the relevant peaks was not possible.[256]

Some doubt must be cast on the practicability of both quantitative and qualitative analysis of amino acids and other organic acids in urine, proposed on the basis of ^{1}H-n.m.r.,[258] though only limited information is offered in the abstracts for these papers. Another conventional use for n.m.r. is for the distinction between tautomers, and 4-nitrohistidine has been shown to exist in solution as the N-1 H form.[259]

Quantitative analysis of ^{13}C-labelled amino acids by n.m.r. has been reviewed.[260] Selective labelling of tryptophan by photo-deuteriation has settled a controversy surrounding ^{13}C-assignments, now established as: C-4, 118.4; C-5, 118.2; C-6, 120.6 ppm.[261]

^{14}N-N.m.r. quadrupole coupling tensors have been determined for crystalline L-histidine hydrochloride monohydrate,[262] and corresponding resonance parameters have been calculated for representative amino acids.[263] More routine applications for ^{15}N-n.m.r. include establishment of a 65:35 E/Z ratio for geometrical isomers of N^{α}-acetyl-N^{in}-nitroso-tryptophan.[264]

5.3 Optical Rotatory Dispersion and Circular Dichroism.- C.d. spectra have been reported for biliverdin-substituted L-amino acids that reveal the formation of bilatriene helices, with c.d. parameters that are very sensitive to the structure of the amino acids involved.[265] While this paper is typical of an application of c.d. that could by now be called classical, the remaining paper for inclusion in this section represents an evolving technique, Raman optical activity spectrometry, for studies of aqueous solutions of L-lysine, L-proline, and L-hydroxyproline.[266] Two specific points of interest arise from these data; interpretation of a strong scissoring vibration mode for lysine and proline, and a significant observation that the Raman optical activity pattern at frequencies greater than 1300 cm^{-1} seems characteristic of the L-configuration.

5.4 Mass Spectrometry.- An idiosyncratic review of the role of m.s. in structure determination of new amino acids from marine algae has appeared.[267]

With all other non-routine papers on m.s. of amino acids located in the appropriate analytical sections later in this Chapter, there remain: molecular-beam studies of the decomposition of glycine in the vapour phase on contact with solid surfaces at 420-800K;[268] secondary-ion emission from amino acids evaporating from metal surfaces[269] or from glycerol solutions containing also a thallium(I) salt;[270] and fast-atom-bombardment MIKE spectra for 20 common L-α-amino acids to provide an order of proton affinities.[271] The solution SIMS study is substantial,

showing that sulpholane is a poor solvent for the purpose and that protonation by trifluoromethanesulphonic acid gives better results than metal ion complex formation.[270] The MIKE study is conceptually simple: the most abundant ion in MIKE of a cluster ion of two different amino acids corresponds to the amino acid with the higher proton affinity. The order that emerges is from glycine (lowest proton affinity) through a generally predictable intermediate order to arginine (highest proton affinity).[271]

5.5 Other Spectroscopic Studies.- Intramolecular hydrogen bonding and formation of hydrogen-bonded dimers is revealed by infrared (i.r.) spectrometry of *N*-acylglycines in solution and dispersed in CsBr.[272] Similar studies of *N*-acetyl glycine, L-alanine, and L-leucine methylamides in chloroform solutions[273] show the co-existence of intramolecularly hydrogen-bonded 5-membered rings and non-hydrogen-bonded species, while i.r. and Raman studies for *N*-acetyl-L-phenylalanine, L-tyrosine and L-tryptophan methylamides have concentrated on solid samples and (for the Raman study) methanol solutions.[274]

Fluorescence spectra of *N*-acetyl-1-pyrenyl-DL-alanine methyl ester reveal intermolecular excimer formation with a smaller rate constant for the dissociation of the D,L-excimer to the locally excited state at room temperature than that for the L,L- (or D,D-) excimer.[275] This confirms earlier work of relevance to theories of prebiotic enantiomeric discrimination.

5.6 Other Physical Studies.- Reviews have appeared on extraction of amino acids from aqueous solutions using reversed micelles [276] and of amino acid transport across liquid membranes.[277] The latter topic is a substantial research area in its biochemical context, for which a representative paper[278] describes L-alanine transport in renal luminal membrane vesicles. There may be physiological relevance to the demonstration of transport of simple monovalent and divalent metal ions and heavy metal ions through $CHCl_3$ membranes by long-chain *N*-acylamino acids.[279]

Solution studies are illustrated further in partition coefficient data for *N*-acetyl dialkylglycinamides[280] and adsorption of valine on a carbon electrode[281] and of alanine or γ-aminobutyric acid on platinum[282] during electro-oxidation.

Thermometric studies include solid-liquid phase transitions of *N*-acylamino acids by differential scanning calorimetry and by i.r. spectrometry,[283] heats of dilution of *N*-acetylalaninamide solutions,[284] and emission thermophotometry of amino acids in a flowing O_2 atmosphere.[285] There is an uncertainty in the results of the latter study, in which it is acknowledged that light emission might arise from oxygenation of unstable pyrolysis products.

Gas-phase electron diffraction data for L-alanine methyl ester are consistent with a *syn*-periplanar N-C-CO torsion angle imposed by formation of a bifurcated intramolecular hydrogen bond between NH_2 and CO groupings.[286]

5.7 Theoretical Studies of Amino Acids.- Molecular-orbital calculations for proline, N-formylprolinamide, and N-acetylprolinamide reveal the formation of an intramolecular hydrogen bond between the acyl oxygen and amide groups that is seen in the peptide γ-turn conformation.[287] Theoretical studies with less obviously applicable results concern calculations of free-energy changes, relevant to aqueous solutions, that accompany the structural changes glycine → alanine and alanine → phenylalanine [288] and calculations of three-dimensional details of the best fit between two interacting amino acids.[289]

6 Chemical Studies of Amino Acids

6.1 Racemization.- This topic can be divided into a number of sub-divisions, one of which uses rate data for racemization of protein amino acids for fossil dating. This has become a controversial subject with doubts being cast on the validity of the results. The uncertainty arises in the role of the immediate environment of the individual amino acid enantiomer that undergoes racemization; while the rate constant data under defined conditions link the degree of racemization with age, it has been found that the process is non-linear (and was shown to be reversed!) from one fossil shell to another, dependent on age and genus of the sample.[290] The deviations for valine and isoleucine racemization are less than for aspartic acid, which has been most commonly used for fossil dating because of its high racemization rate constant (see also ref.420).

The isoleucine:alloisoleucine ratio for snail shells from Holocene sediments of the northern Negev desert is a useful index of age variations within a layer, but not for absolute chronology, for which the ^{14}C technique was used.[291] Racemization of bone collagen has been subjected to a laboratory study, showing that within the time-scale of a practicable research project the onset of racemization can only be detected at elevated temperatures (>40°C).[292] The topic of amino acid dating has been reviewed.[293]

Strong basic anion exchange resins in the OH^- form partly neutralized with 5-sulphosalicylaldehyde have been found to bring about racemization of amino acids in the presence of a copper(II) salt at a faster rate than the well-known equivalent solution reaction.[294] Leucine, phenylalanine and tryptophan amides undergo the equivalent racemization process in the presence of their parent amino acids and formylated phenol - formaldehyde resins or polystyrene - divinylbenzene - salicylaldehyde condensation products.[295] A similar-looking process in which L-methionine undergoes racemization in acetic acid containing a catalytic amount of salicylic acid[296] may have another mechanistic explanation, since an N-acetylamino acid is known to undergo racemization in the presence of acetic anhydride. The other well-known Schiff-base-mediated racemization procedure employing pyridoxal has been shown to be accelerated by phosphate ion.[297]

Certain N-protected α-amino aldehydes are prone to racemization during chromatography on silica gel, though bulky N-[9-(9-phenylfluorenyl)]-L-alaninal has been shown to be configurationally stable in this respect and in the presence of some simple reagents.[298]

6.2 General Reactions of Amino Acids.- This substantial section collects reports on reactions in which amino and carboxy groups and their simple derivatives are involved, either together or separately. In the latter category, the emphasis is on papers of wider interest than the synthesis of protected amino acids for peptide synthesis.

Carpino has reviewed the chemistry of Fmoc and related base-sensitive N-protecting groups that are useful in the amino acid and peptide field.[299] Reagents explored for introduction of the Fmoc (using Fmoc N-hydroxysuccinimide ester[300]) and other standard N-protecting groups include pentafluorophenyl formate (for N-formylation),[301] BocN=C(CN)Ph for the synthesis of Boc-L-phenylalanine,[302] crosslinked polystyrene carrying CF_3COSCH_2- or $CF_3CO_2CH_2$- groups for N-trifluoracetylation,[303] and 1-(N-benzyloxycarbonyl)benzotriazole and dibenzyl dicarbonate for Z-amino acid formation.[304] The use of the alternative reagent for the synthesis of Fmoc derivatives[300] minimizes the formation of Fmoc-dipeptide side products. Unwanted side reactions have also been noted in the reaction of palmitoyl chloride with L-cystine di-t-butyl ester, giving some N-$Me(CH_2)_{13}COCH[(CH_2)_{13}Me]CO$-substituted side products,[305] and in the formation of different condensation products between an amino acid and samples of glutaraldehyde of varying quality.[306] One of these is assigned the somewhat unlikely structure (45), based on the propensity of Schiff bases to participate in aldol-type reactions.[306]

Trimethylsilylation of amino acids in the presence of CO_2 giving trimethylsilyl derivatives of N-carboxyamino acids,[307] copper(II)-promoted acylation of leucine with a β-ketothiolester,[308] and N-alkylation of amino acid esters with 5-methoxymethylene-1,3-dioxan-4,6-dione ("methoxymethylene Meldrum's acid") to give (46)[309] have featured in recent papers. Asymmetric hydrogenation of N-pyruvoyl-L-proline esters gives N-(S)-lactoyl analogues with diastereoisomeric excesses of up to 59%.[310] D-α-Hydroxy acids have also been prepared from L-α-amino acids via halogeno-acids (formed using $NaNO_2$/HX).[311]

Kinetics of nitrosation of thioproline have been determined.[312]

Catalytic hydrogen-transfer deprotection of N-benzyl-[312] and N-Z-amino acids[313] using ammonium formate and 10%Pd-C is rapid at reflux temperatures. The formation of adenine in heated solutions of N-(4-purinyl)glutamic acid containing a copper(II) salt is a notable C - N cleavage reaction.[315] A curious C - C cleavage process is seen in the formation of Z-β-alanininamide as a result of an attempted Hofmann-type rearrangement of 1-aminocyclopropanamide using

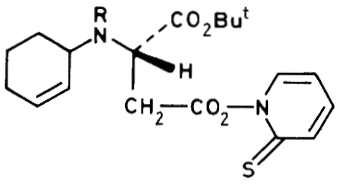
$PhI(OCOCF_3)_2$.[316]

Esterification of amino acids using a mono-alkyl sulphate[317] and an optimized route to L-histidine benzyl ester ditosylate[318] have been reported. Esterification of N-protected amino acids catalyzed by papain[319] (only the α-carboxy group of aspartic and glutamic acid derivatives is esterified) is successful with Boc-amino acids and a wide range of alcohols and diols in a two-phase procedure.[320] Details of the preparation of 4-(Boc-aminoacyloxymethyl)phenylacetic acids for making PAM resins for solid-phase peptide synthesis have been described.[321] Esterification of N-protected amino acids and peptides, but using 15-crown-5 in DMF with 1M sodium hydroxide and an alkyl halide, is claimed as a mild method.[322] However, the method involves distilling off water (from a mixture containing sodium hydroxide) and some DMF prior to adding the alkyl halide, and then the mixture is kept at 40°C for 2 hours before evaporation, so there seems to be considerable scope for racemization and side reactions. 1,2,2,2-Tetrachloroethyl esters are formed from N-protected amino acids and an alkyl 1,2,2,2-tetrachloroethyl carbonate.[323]

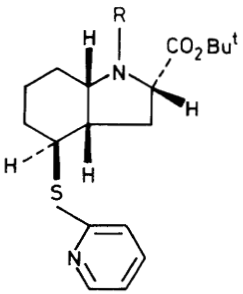
Activation of an N-protected amino acid through mixed-anhydride formation with isopropenyl chloroformate permits easy esterification by alcohols.[324] Mixed anhydrides formed from N-alkoxycarbonylamino acids[325] can undergo intramolecular acylation of the urethane nitrogen atom as a side reaction during aminolysis by an amino acid[326] or by an amino acid ester.[327] Similar acyl transfer is seen in the conversion of α-enamino acid anhydrides (47) to corresponding acylamino acids (48) under the influence of aqueous hydrochloric acid.[328] The same process operates in the reaction of a chloro-imine with an N-alkoxycarbonylamino acid [(49) → (50)], previously thought to lead to the N-phenyl-N-benzylamide.[329]

Reactions of carboxy-group derivatives of amino acids include Friedel-Crafts acylation by N-protected L-prolyl chlorides giving corresponding aryl ketones,[330] oxidative decarboxylation of N-methoxycarbonyl-[O-(t-butyldimethylsilyl)]-L-hydroxyproline to give the corresponding 5-methoxypyrrolidine by electrolysis in MeOH,[331] a new route to α-amino aldehydes through $LiAlH_4$ reduction of a Z-amino acid 3,5-dimethylpyrazolide,[332] and conversion of Z-D- or L-alanine into corresponding trans-2,5-dimethylpyrrolidine enantiomers via $ZNH\text{-}CHMe\text{-}CH_2CH_2CH{=}CH_2$.[333] Oxazolin-5-ones are a form of activated ester, and continuing interest in their oxidative cleavage into acylamides (see also Vol.19, p.29) is shown in O_2 - Pd peroxidation[334] and in radical anion formation with K or KO_2.[335]

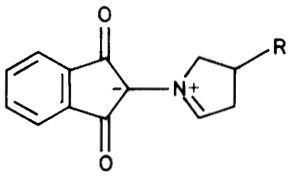
Ever more numerous papers reflect the considerable interest in enantioselective hydrolysis of N-acylamino acid p-nitrophenyl esters, either involving micellar media containing an L-histidine-containing peptide[336,337] or imidazole in the presence of a chiral surfactant,[338] or involving α- or β-cyclodextrins.[339] One of these papers[337] records the "perfectly enantioselective hydrolysis" of N-dodecanoyl-D- and L-phenylalanine p-nitrophenyl esters in micelles containing Z-L-Phe-L-His-L-Leu-OH.



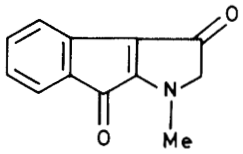
(51)



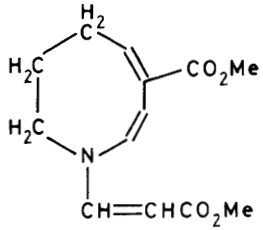
(52)



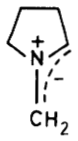
(53) R = H or OH



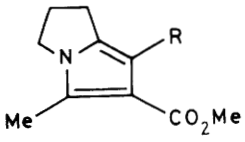
(54)



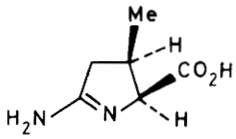
(55)



(56)



(57)



(58)

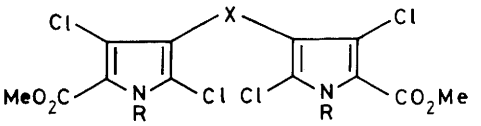
Representative papers for the many mechanistic studies that have appeared this year for oxidation of individual amino acids describe bromamine-T/glutamic or aspartic acid,[340] alkaline hexacyanoferrate(III)/tryptophan,[341] and chloramine-T/alanine, 2-aminobutyric acid, valine, serine and threonine.[342] Chloramine-T oxidation of arginine in acid media is catalyzed by Cl^- (which is thought to become Cl^+ in the oxidizing medium).[343] Since one of these papers[342] describes a 're-investigation' of the well studied chloramine-T system, there will no doubt continue to be expenditure of much effort on this topic.

Uses of amino acids as chiral auxiliaries in asymmetric synthesis are cited elsewhere in this Chapter; interesting synthetic developments in nitrogen-heterocyclic chemistry starting with amino acids continue to call for space in this Section. Maillard condensation of [1- or 2-^{13}C]glycine or [^{15}N]glycine with D-xylose or its [1-^{13}C] isotopomer leading to melanoidins (structural details not established) is a paper[344] of an important research topic. The generation of a carbon-centred radical from an N-hydroxypyridine-2-thione ester has been studied in the context of the L-aspartic acid derivative (51), leading to the condensed proline (52) and its diastereoisomer.[345] Azomethine ylides are intermediates in the formation of oxazolidines from secondary amines and aldehydes (see Vol.19, p.29) and include the yellow ninhydrin - proline (or hydroxyproline) condensation product ((53); reaction in ethanol at 0°C), recognised for the first time to be a stable azomethine ylide.[346] In contrast, sarcosine and ninhydrin give (54).[347] Further work on this enlightened view on the mechanism of the Strecker decarboxylation of amino acids covers the formation of the ring-expanded proline (55) arising via the azomethine ylide (56) through the reaction of proline, formaldehyde, and methyl propiolate.[348] A full paper has appeared on cycloaddition of proline to methyl 2-alkynoates in acetic anhydride, leading to pyrrolizines (57).[349] The adduct from the intermediate mesoionic oxazolinone, formed from N-acetylproline by the action of acetic anhydride, yields the final product through retrocycloadditive decarboxylation.

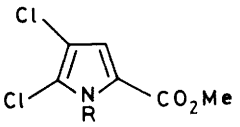
A representative paper[350] on the formation of β-lactams from β-amino acids describes the use of bis(5'-nitro-2'-pyridyl)-2,2,2-trichloroethyl phosphate for the purpose.

6.3 Specific Reactions of Amino Acids.- The literature on chemical studies that result in side-chain modifications to amino acids is reviewed in this Section. Readers seeking a full overview should also read both the preceding section and the earlier sections covering synthesis (where papers describing the use of one amino acid to synthesise another are mostly located).

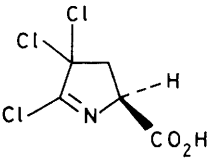
There are relatively few reactions in which changes to aliphatic amino acid hydrocarbon side chains are accomplished without affecting the amino and carboxy functions or the α-carbon atom, but suitably protected examples undergo γ-proton



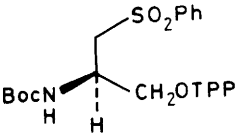
(59) X = S, SO, SS, or SO_2



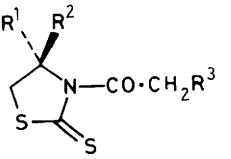
(60)



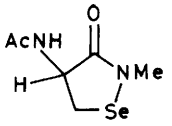
(61)



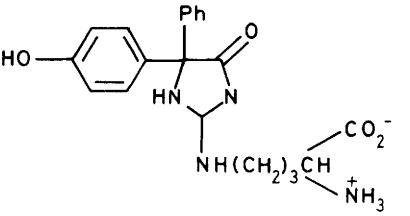
(62)



(63)



(64)



(65)

abstraction by hydroxyl radicals (from $TiCl_3$ - H_2O_2) rather than the more common α-attack.[361] 1-Aminocyclopropanecarboxylic acid, not surprisingly, reacts more readily, and its biological role as ethylene source has been modelled using the O_2 - Mn^{2+} - 1-benzyl-3-carbamoyl-1,4-dihydropyridine - flavin mononucleotide system.[362]

Oxidation of N-alkoxycarbonylprolines gives the corresponding pyroglutamates,[363] which have a role in a synthesis of chiral pyrroline-5-carboxylic acids (58).[364] Aspartic and glutamic acids are perhaps the most hard-worked of the protein amino acids as chiral starting materials in synthesis, a further example (see also, inter alia, refs.96, 116) being a three-step route from L-aspartic acid to N-trityl-L-homoserine lactone (via selective reduction in 50-60% yield of the N-trityl dibenzyl ester using DIBAH).[365] A convenient preparation of L-aspartic β-semialdehyde from L-methionine via its sulphonium salt, and pyridinium chlorochromate oxidation of the derived homoserine, leads on to other synthetic possibilities [Wittig elaboration to L-2-amino-hept-4,6-dienoic acid; a new synthesis of bulgecinine (see also ref.121)].[366]

Uranyl-sensitized photo-oxidation of aspartic acid in aqueous acidic media gives malonic acid.[367] This amino acid is included with lysine and glutamic acid in an e.s.r. study of free-radical formation on pyrolysis through the temperature range 473-873K.[368] More routine results, useful however in synthesis, concern the preparation of β-aspartates and γ-glutamates from esterification of the amino acids by alkanols in the presence of Na_2SO_4 and HBF_4,Et_2O [369] and the formation of the same mono-esters through alcalase-catalyzed hydrolysis of the di-esters.[370] L-Glutamic acid is the source of (3S)-pyrrolidinol, via L-(-)-$H_2NCH_2CH_2CH(OH)CO_2H$.[371] Pyrroles are formed from pyroglutamate esters and thionyl chloride [which leads to (59)] or PCl_5 [which leads to (60) and the 3,3-dichloropyrroline (61)].[372] All three functional groups of DL-threonine are modified by PCl_5 in dioxan to give $MeCHClCH(COCl)N{=}PCl_3$, and in benzene the product is $Cl_2P(O)OCMe{=}CHCOCl$.[373] The hydroxy group in serine is modified in an enantiospecific synthesis of representative β-alkylated alanines (D- and L-homophenylalanine, norvaline, and norleucine), in which Boc-L-serine is converted into the phenylsulphonylmethyl derivative ((62) and its enantiomer) by routine methods.[374] Activation and alkylation of the α-methylene grouping permits introduction of a chosen β-substituent. Removal of the tetrahydropyranyl protecting group followed by oxidation (pyridinium chlorochromate) converts the original serine side chain into the carboxy group of the target amino acid.[374] Stereoselective alkylation of benzene with N-phthaloyl-(2S,3R)-threonine O-trifluoromethanesulphonate (in CF_3SO_3H at 80° during 10 h) gives N-phthaloyl-(2S,3S)-3-methylphenylalanine,[375] while the (2S,3S)-threonine derivative gives a 40:60 mixture of (2S,3R)- and (2S,3S)-diastereoisomers in the same reaction.[375]

The hydroxy and carboxy groups of N-palmitoyl-hydroxyproline can be condensed intermolecularly to form novel polyesters,[376] and a similar approach based on O-

cyanylation of tyrosine followed by poly(iminocarbonate) formation is described in the same paper. Attack at aromatic and heteroaromatic groupings feature in a study showing the relatively greater propensity of the tyrosine rather than the tryptophan side chain to undergo free-radical hydroxylation (by ClO_2 or NO_2 in alkaline aqueous media, in which HO$^{\bullet}$ is generated).[367] L-Tyrosine offers a starting point for synthesis of substituted dopas through Friedel-Crafts acetylation, reduction of the acetyl group, and rearrangement.[368] $S_{RN}1$ Reactions of protected *S*-mercapto-phenylalanines and -di-iodotyrosines to give phenylthio analogues have been described.[369] The phenyl moiety of phenylalanine is unchanged through reaction of *N*-protected esters with 4-t-butyliodylbenzene, 4-$Bu^tC_6H_4IO_2$, whereas the familiar oxidative changes for tyrosine (→ dopaquinone), tryptophan (→ kynurenine), and histidine (→ γ-formamidoglutamine) are seen for the heteroaromatic amino acids.[370] Conversion of tryptophan into aspartic acid (and of valine into isobutyric acid, and phenylalanine into phenylacetic acid) is the outcome of ruthenium(VIII) oxidation (via $RuCl_3$).[371] Degradation of tryptophan into α-amino-β-carboxymuconate ε-semialdehyde by nicotinamide enzymes is followed by non-enzymic condensation into pyridine-2,3-dicarboxylate in aqueous media at pH > 4.5.[372] D-Tryptophan (formed from the L-enantiomer) is the biosynthetic source for indole-3-acetic acid in *Pisum sativum*.[373]

Conflicting advice about the stability and optimum storage conditions for *S*-adenosyl-L-methionine has been clarified through a study suggesting that epimerization and other processes do not occur in solutions at pH values 3-5.[374] Several papers have appeared on sulphur-containing amino acids (see also ref.3) dealing with conversion of *S*-carboxymethyl-L-cysteine into a diastereoisomeric mixture of sulphoxides with H_2O_2,[375] kinetics of HS^-/cystine reduction and competitive processes,[376] $Br_2/^{18}OH_3^+$ oxidation of L-cystine to cysteic acid (all oxygen atoms are labelled, revealing the intermediacy of a sulphenic-carboxylic anhydride in this reaction),[377] and synthesis of a useful chiral auxiliary thiazolidinethione (63) from L-cysteine (analogous to the oxazolidinethione from L-serine) for use in boron- and tin-mediated aldol reactions (see Section 4.2).[378] Another study of u.v. photolysis of aqueous L-cysteine (254 nm/24 h) has been reported, in which 36 volatile products extractable into dichloromethane were identified.[379] 2-Methyl-thiazole predominated in the group of 5-membered heterocyclic compounds in the product mixture.

Selenocysteine has been converted via its *N*-acetyl-Se-benzyl methylamide into the cyclic seleninamide (64), adding to knowledge of selenium functional-group interconversions.[380]

The guanidine side chain of arginine hydrochloride has been condensed with 4-hydroxybenzil to give the imidazolinone (65).[381]

Introduction of protecting groups is either intended to select for a side-chain function, as in the synthesis of *S*-Fmoc-L-cysteine (deprotection by 50% piperidine in DMF),[382] *S*-acetamidomethyl-*N*-Fmoc-L-cysteine (introduced using $AcNHCH_2OH$,

prepared from $AcNH_2$ + HCHO in aq. KOH),[383] N$^{\omega}$-2,2,5,7,8-pentamethyl chroman-6-sulphonyl-L-arginine (cleavable by 50% trifluoroacetic acid in CH_2Cl_2), [384] and N$^{\tau}$-alkylation of N$^{\tau}$-phenacyl-histidine (removal of the phenacyl group with Zn/AcOH),[385] or is found, unexpectedly, to involve the side chain, as in the formation of N-Fmoc pyroglutamic acid during the reaction of FmocCl with glutamic acid.[386]

6.4 Non-Enzymic Models of Biochemical Processes involving Amino Acids.- Specific interactions of anticodon dinucleoside monophosphates with cognate amino acids, as detected by precise u.v. difference absorbance spectroscopy, are of similar magnitude to base-base stacking interactions.[387]

6.5 Effects of Electromagnetic Radiation on Amino Acids.- The emphasis on photolysis and fluorescence studies for tryptophan continues to provide most of the material for this Section, including haematoporphyrin-sensitized 630nm pulsed laser[388] and related[389] photo-oxidation studies. Fluorescent light irradiation of tryptophan in the presence of riboflavin gives kynurenine and its N-formyl derivative, most rapidly at pH 7.5,[390] as seen also in irradiation in the presence of "oxidizing methyl linoleate"[391] (cf. Vol.19, p.29) and of selectively sulphonated gallium phthalocyanines.[392] Photosensitized oxidation of N-acetyltryptophanamide in neutral aqueous solutions in the presence of various dyes has been shown to involve only singlet oxygen.[393]

The one-electron reduction potential of the oxidized tryptophan radical at neutral pH is greater than that of the oxidized tyrosine radical.[394] Photoinduced electron transfer to cytochrome c from kynurenine and its N-formyl derivative is revealed in reduction under both aerobic and anaerobic conditions.[395]

Fluorescence behaviour of tryptophan has been reviewed.[396] The intermolecular equivalent of one of the tryptophan quenching mechanisms, the fluorescence quenching of indoles by simple amino acids, has been studied from the point of view of the effect of either anionic or cationic micelles.[397]

There is a range of radioprotective and radiosensitizing effects for *E.coli* and mice, shown by sulphur-containing amino acids.[398] Further studies of 265nm laser flash photolysis of dopa and its 5S-cysteinyl and 2,5-S,S-dicysteinyl derivatives have been reported.[399]

7 Analytical Methods

Current methodology in all aspects of amino acid analysis has been reviewed.[400]

7.1 Gas-Liquid Chromatography.- A three-volume set[401] contains thorough coverage of amino acid analysis by g.c. Detailed case studies are included to illustrate the general trends towards derivatization using N(O)-perfluoracylated amino acid

alkyl esters, trends also revealed in the current primary literature.

N-Trifluoroacetyl n-butyl esters have been chosen for the derivatization protocol for analysis of representative amino acids[402–405] and specifically for [1-^{15}N-5,β,β-$^{2}H_3$]- and [1,3-$^{15}N_2$-5,α,β,β-$^{2}H_4$]-N^{im}-carbethoxyhistidine.[406] The purpose of a particularly detailed study[403–405] using these derivatives was to clarify long-standing anxieties concerning artefacts and reliability in g.c. analysis of amino acids. Careful attention to aspects of a protein hydrolysis regime is recommended;[403] if this advice is heeded, excellent agreement with results of ion exchange amino acid analysis is generally achieved.[404,405] N-Heptafluoroisobutyroylamino acid isobutyl esters continue to feature regularly in the literature,[405] and a substantial review of their analytical use has appeared.[407] G.c.-m.s. analysis of 4-hydroxyproline and 5-hydroxypipecolic acid in brain and blood samples using their heptafluoroisobutyroyl methyl esters is applicable at 3-6 nmol cm^{-3} and 20-30 pmol cm^{-3} levels, respectively.[408]

Silylated amino acids have been considered to be less reliable for the present purpose, but the discovery that N(O)-dimethyl-t-butylsilylated amino acids possess satisfactory stability under both preparative and g.c. conditions has led to renewed interest in them.[409–412] One of these studies[410] involves g.c.-m.s. analysis of cysteine, methionine, and homocysteine in serum samples, and another[411] also grasps the nettle of their use for the analysis of the polyfunctional amino acids, lysine, arginine, and histidine. Introduction of the dimethyl-t-butylsilyl moiety into these amino acids using N-methyl N-(dimethyl-t-butylsilyl)trifluoroacetamide by reaction at 150° during 2.5 h does not cause multiple silylation because of the bulk inherent in the group being introduced (the usual reaction protocol involves reaction in MeCN at room temperature).[411] However, two peaks have been reported for g.c. of arginine derivatized in this way,[412] and further refinement of derivative preparation is needed.

Simple acylated derivatives continue to be used in some cases,[413–415] such as N-acetylamino acid n-propyl[413] or isopropyl[414] esters, and these studies include comparisons with perfluorinated analogues. Treatment of a peptide with t-butyl isocyanate and subsequent N-terminal cleavage with HCl in isopropanol provide a new use for g.c. with the oldest N-terminal amino acid analysis method and gives both structure and configuration of the N-terminal residue when Chirasil® (a chiral stationary phase) is employed for the g.c. analysis.[416] Estimation of enantiomeric excesses through this form of "chiral g.c." continues to be developed, with an example closely related to that just described. Transesterification of L-proline benzyl ester with isopropanol and formation of the ureide is followed by g.c. over Chirasil-Val® for enantiomeric analysis of the product.[417] Further examples[418–420] of the use of these commercial phases include glass capillary g.c. of N-acylamino acid isopropyl esters [419] and an application to dating of quaternary mammal fossil teeth based on enantiomer ratios for the eleven amino acids identified in these samples (particularly concentrating on the

D:L ratio for aspartic acid; see also Section 6.1).[420] Exploration of new chiral g.c. phases has been reported through a study of the separation of *N*-trifluoroacetyl-DL-amino acid isopropyl esters over immobilized (*S*)-*N*-phenyl-5-isopropylthiohydantoin,[421] a stationary phase which would normally have been thought to be difficult to prepare in enantiomerically pure form. The alternative approach to g.c. resolution involves creation of diastereoisomeric pairs of derivatives. *N*-Trifluoroacetyl menthyl esters[422] have been used in a study of the D-amino acid content of single-cell proteins, showing that significant amounts are found only for alanine and glutamic acid residues.[423] Further exploration of the variation of this approach in which the partially racemic sample is converted into its *N*-trifluoroacetyl-L-prolyl derivative has been reported.[424] A g.c. study of the conversion of D/L-aspartic acid mixtures into their isoindoles through reaction with *o*-phthaldialdehyde and *N*-acetyl-L-cysteine (a method that has shown promise in the equivalent h.p.l.c. process) concludes that it is superior in this application to the more usual chiral-phase g.c. resolution of *N*-trifluoracetyl isopropyl esters.[425]

7.2 Ion Exchange Chromatography.- Assessment of an improved amino acid analyzer that uses 3μm resin beads and can detect to 10 pmol (using ninhydrin) or 500 fmol levels (using fluorescamine) has been reported.[426] Aspects of existing commercial methodology are being assessed single-handedly,[427-431] for factors that accelerate deterioration,[427] including an alternative solvent for flushing ninhydrin,[428] and the performance of the integrator.[429] A non-instrumental objective is contained in one of these papers, a spurious peak in histidine analyses that is concluded to have been introduced by the buffer.[430]

Application of the Beckman amino acid analyzer for the determination of cystine, cysteine-penicillamine disulphide, and pipecolic acid has been described.[432]

The effect of n-propanol in the cation exchange separation of amino acid esters has been assessed.[433]

7.3 Thin-Layer Chromatography and Related Separation Methods.- Standard applications of t.l.c. have been described in separations of dansylamino acids,[434,435] including nanomole analysis of neurotransmitter amino acids [435] and picomole separations of dansylamino acid methyl esters.[436] Analysis of phenylthiohydantoins by t.l.c. over silica impregnated with zinc, cadmium, and mercury salts,[437] with transition metal ions,[438] or with (+)-tartaric acid[439] has been investigated.

Cysteine and cystine show up as yellow spots on t.l.c., when sprayed with Ellman's reagent [5,5'-bis(dinitrobenzoic acid) disulphide], a useful distinction between the two being the slow time for revelation of the colour with cystine (the cysteine colour is rapidly formed).[440] Cation exchange foils have been used in

t.l.c. of ^{15}N-labelled amino acids.[441] Another less-commonly used variation is over-pressured layer chromatography, which carries a number of advantages in terms of speed and resolution, e.g. in separations of sulphur-containing amino acids.[442]

Calculations of relationships between t.l.c. retention data with amino acid structure have appeared.[443]

Paper chromatography rarely gains an entry to the literature these days, but it has its uses, such as for the analysis of diaminopimelic acid in bacterial cells.[444] Paper electrophoresis offers improved identification of opines (nopaline and octopine) in plant tissues, these N-carboxyalkylamino acids being revealed by u.v.-fluorescent red-purple products formed with phenanthrenequinone.[445]

7.4 High-Performance Liquid Chromatography.- Reviews include a chapter on h.p.l.c. of amino acids and peptides in a useful small book,[446] another on amino acids in a larger treatise,[447] and amperometric detection methods.[448]

Supercritical liquid CO_2 h.p.l.c. is a fundamentally novel approach that is being widely explored in analysis and has been shown to give fast, efficient separation of N-acetylamino acid t-butyl esters on a chiral stationary phase.[449]

H.p.l.c. separation of amino acids and their post-column derivatisation is favoured only by a minority of h.p.l.c. practitioners, though there are benefits associated with the delivery of amino acids free from interfering species into the detector. There are interesting possibilities for pre-column purification or concentration of amino acid samples. The use of a short boronate affinity column for purifying samples before passage to an h.p.l.c. column (estimation of 5-S-cysteinyldopa in urine) [450] and enrichment of tryptophan and tyrosine by pre-column silica bis(thiocarbamate) treatment of samples to which a copper(II) salt had been added [451] are representative of these approaches.

Separation of underivatized amino acids using the ion pair (or "ion interaction") principle, in which the buffer contains a long-chain alkanesulphonic acid,[452] has been compared with discrimination based on crown ether complexation.[453] A related method employing copper(II) alkanesulphonates[454] seems to offer improved resolution. The requirements for the mobile phase in the separation of underivatized amino acids need, and will undoubtedly receive, further study.

The detection of amino acids emerging in such media from the h.p.l.c. column imposes no special problems, and electrochemical methods have been described for cysteine[455] and subnanomole analysis of other amino acids.[456] Estimation of serum methionine[457] using an anodically treated glassy carbon electrode illustrates the continuing efforts towards the creation of tailor-made analytical packages. Post-column o-phthaldialdehyde-mercaptoethanol derivatization of amino acids separated using a sodium alkanesulphonate buffer is typical of the standard general approach in this category of h.p.l.c. amino acid analysis,[458] including a use for the

analysis of total hydrolysates from samples of polyacrylamide gel carrying 5μg or more of protein.[459] The gel + protein sample is hydrolysed in the usual way, with thioglycollic acid added to prevent degradation of tyrosine, cysteine, and methionine.

Brief mention can be made of estimation of tryptophan (219nm absorption),[460] N^{G}-hydroxymethyl-L-arginines (as newly discovered amino acids in serum in urine arising from reaction with endogenous formaldehyde),[461] tyrosine and phenylalanine in dried blood spots after extraction with ethanol,[462] and desmosine and isodesmosine in elastin[463] and other tissue hydrolysates.[464-466] Methylated amino acids and 5-hydroxylysine are also included in one of these reports,[464] which provides picomole level data.

Pre-column derivatization, while requiring more reference citations, needs only limited space here because most of the papers build upon methods that are becoming standard. Out of the ordinary, however, is an assay for N-acetyl-S-(N-alkylthiocarbamoyl)-L-cysteine, the principal plant metabolite from an alkyl isothiocyanate.[467] The process by which the metabolite is formed is reversed for the purpose of analysis, and the resulting isothiocyanate is derivatized into a thiourea using butylamine.

Dansylamino acids[468-470,495] and their dabsyl analogues[471,472] continue to be advocated for routine amino acid analysis. In a comparative study,[472] results for the latter derivatives are shown to be in good agreement with those employing the o-phthaldialdehyde - mercaptoethanol procedure, which is among the most widely used derivatization regimes at the moment.[473-485] Standard applications of the last-mentioned method to 3-methylhistidine,[473,474] 1-aminocyclopropanecarboxylic acid,[475] N^{ε}-trimethyllysine,[476] branched-chain α-amino acids,[477] and samples containing large numbers of amino acids[478] have been described. Papers on this topic with specific attention to practical aspects deal with resolution (narrow-bore versus normal column h.p.l.c.),[479] improved gradient elution,[480] and automated o-phthaldialdehyde-mercaptoethanol analysis.[481]

The other burgeoning application of h.p.l.c. in amino acid analysis concerns pre-column phenylthiocarbamoylation using phenyl isothiocyanate.[486-494] Artefacts arise during (and subsequent to) this operation, and it has been found that ether extraction of the derivatized sample prior to h.p.l.c. leads (paradoxically) to "fewer losses".[486] The literature continues to describe applications of this method, which can now be called routine,[487-490] including estimations of 4-hydroxyproline,[487] phenylalanine and tyrosine.[489] The method has been extended successfully to more problematic cases, phosphorylated[491,492] and sulphated[492] amino acids, N-mono- and N,N-di-methylarginines, methylated lysines, and 3-methylhistidine.[493] The phosphoserine content of proteins[491] is not actually estimated as such, but as S-ethylcysteine (formed by operating on the protein, hydrolysis, and phenylthiocarbamoylation). The tryptophan content of proteins can be estimated by this method without interference from the 0.4% mercaptoethanol

that is routinely included in the 6M hydrochloric acid hydrolysis medium.[494]

There are relatively few papers covering h.p.l.c. analysis of phenylthiohydantoins in non-routine contexts. Among these,[495-498] chemical ionization m.s. studies[496] and analysis at 20-100 fmol levels[498] are featured. There are additions to the experience accumulated over several years of the use of fluorescent derivatives formed with 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole[499] and its 7-fluoro-4-sulphonate analogue.[500] Fmoc derivatives offer several advantages for h.p.l.c. analysis, being introduced under mild conditions and showing readily measurable fluorescence at 313nm (excitation at 260nm), allowing pmol level detection.[501,502] A new form of derivative links the chemiluminescent phthalhydrazide group to the amino group of an amino acid through an isothiocyanate group, and can be detected at the level of 10 fmol per 20μl injected sample.[503] A specific fluorescence-forming reaction between dopa and 1,2-diphenylethylenediamine permits detection at 10 fmol levels.[504]

Estimation of enantiomer ratios using h.p.l.c. continues to develop along lines that have become established over several years. The o-phthaldialdehyde procedure using a chiral thiol is represented in several papers,[505-509] employing N-acetyl-L-cysteine[505-507] in most cases (but 2,3,4,6-tetra-O-acetyl 1-thio-β-D-glucopyranoside has been tried in one study, establishing a fmol level assay[509]). The other main "chiral mobile phase" approach employs the copper(II) - L-proline system for enantiomeric analysis of α-alkyl-lysines and -ornithines (but not ornithine itself)[510] and for simple α-amino acids (but not for γ-aminobutyric acid)[511] or the copper(II) - $(CH_2)_n(L\text{-}NHCHRCO_2H)_2$[512] and simpler copper(II) - L-amino acid systems[513,514] for the resolution of dansylamino acids[512,513] and o-phthaldialdehyde-mercaptoethanol - DL-amino acid condensation products.[513] Chiral stationary phases made in the simplest way by adsorbing a protein on to silica[515] (cf.ref.228) (for the resolution of N-benzoyl-DL-amino acids) or N-alkoxycarbonyl (S)- or (R)-naphthylethylamides[516,517] or related α-arylglycine derivatives[518] or proprietary products ('Chiral-Pak WH'®)517 represent the other major approach.

Comparison of four systems, o-phthaldialdehyde - N-acetyl-L-cysteine, β-cyclodextrin-containing mobile phase, D-phenylglycine-based stationary phase, or Chiral-Pak WH, leads to the conclusion that each has its virtues (and each has its shortcomings).[517]

7.5 Other Analytical Methods.- A multicompartment apparatus for isoelectric-focussing analysis of amino acids has been described.[519]

7.6 Estimation of Specific Amino Acids.- The title of this Section would suggest enzymic methods, but individual amino acids can also be analysed without interference from others by several classical chemical methods.

Oxidative de-amination of branched-chain α-amino acids (valine, leucine, or isoleucine) by leucine dehydrogenase is quantitatively linked to fluorescence

generated by NADH that is part of the reagent.[520] A similar approach but a different physical basis is involved in an L-lysine assay using L-lysine decarboxylase immobilized on a CO_2 sensor,[521] in an L-glutamic acid assay using immobilized L-glutamine synthetase/NH_4^+/ATP (based on the change in pH due to H^+ liberated in the transformation),[522] or conversely in an L-glutamine assay employing an immobilized glutaminase/glutamate oxidase electrode.[523]

γ-Carboxyglutamic acid has been estimated in urine using capillary isotachophoresis.[524]

Colorimetric methods are represented in the estimation of citrulline and N-carbamoyl-β-alanine [525] and spectrophotometric assays for cysteine (as its methylglyoxal derivative)[526] and cystine.[527] Serine, formed from sarcosine as a result of oxidative demethylation by Pseudomonas WRE, can be estimated by conversion into formaldehyde (arising from the β-carbon atom through the Nash reaction) by spectrophotometric methods.[528]

Cystine gives a linear response down to about 10^{-8}M levels in hydrodynamic modulation voltammetry.[529] G.c. estimation of selenomethionine can be based on the quantitative character of the cyanogen bromide reaction that leads to MeSeCN with this amino acid.[530]

REFERENCES

1 "Asymmetric Synthesis: Construction of Chiral Molecules Using Amino Acids", G.M.Coppola and H.F.Schuster, Wiley, New York, 1987.

2 "α-Amino Acids, N-Carboxyanhydrides, and Related Heterocycles; Synthesis, Properties, Peptide Synthesis, and Polymerization", H.R.Kricheldorf, Springer Verlag, Berlin, 1987.

3 Methods in Enzymology, 1987, Vol.143. Representative Chapter contents: T.Ubuka, 17 (Reactions of S-(2-Amino-2-carboxyethylsulphonyl)-L-cysteine leading to S-sulpho-L-cysteine and L-alanine sulphodisulphane); J.D.H.Cooper and D.C.Turnell, 141 (Cysteine and Cystine); F.J.Leinweber and K.J.Monty, 160 (Cysteinesulphinic acid); R.K.Gordon, G.A.Miura, T.Alonso, and P.K.Chiang, 191 (S-Adenosyl-L-methionine); J.H.Fellman, 223; K.Soda, 453 (Microbial metabolism of sulphur-containing amino acids).

4 "Amino Acids in Health and Disease: New Perspectives", UCLA Symposia in Molecular and Cell Biology, New Series, Vol.55, Ed. S.Kaufman, A.R.Liss Inc, New York, 1987.

5 "Escherichia coli, Salmonella typhimurium", edited by F.C.Neidhardt, American Society of Microbiology, Washington DC, 1987, Vol.1. Reviews of biosynthesis of protein amino acids by: N.Glansdorff, 321 (arginine); T.Leisinger, 345 (proline); H.E.Umbarger, 352 (leucine, isoleucine); M.E.Winkler, 368 (histidine); A.J.Pittard, 368 (phenylalanine and tyrosine); G.V.Stauffer, 412 (serine and glycine); N.M.Kredich, 419 (cysteine); G.N.Cohen and I.Saint-Girons, 429 (threonine, lysine and methionine), etc.

6 J.R.Cronin, S.Pizzarello, G.U.Yuen, S.Epstein, and R.V.Krishnamurthy, Nature, 1987, **326**, 477.

7 S.Epstein and E.Zinner, Earth Planet.Sci.Lett., 1987, **84**, 359.

8 T.Wakamiya and T.Shiba, Kagaku, Zokan (Kyoto), 1987, 121.

9 R.Ruembeli, F.Suter, M.Wirth, W.Sidler, and H.Zuber, F.E.B.S. Lett., 1987, **221**, 1.

10 A.V.Klotz and A.N.Glazer, J.Biol.Chem., 1987, **262**, 17350.

11 W.H.Schaefer, T.J.Lukas, I.A.Blair, J.E.Schultz, and D.M.Watterson,

J.Biol.Chem., 1987, **262**, 1025.
12 A.N.E.Birch, L.E.Fellows, and K.Doherty, Phytochemistry, 1986, **25**, 2745.
13 T.Ohyama, Nippon Dojo Hiryogaku Zasshi, 1986, **57**, 300 (Chem.Abs., 1987, **106**, 135303).
14 R.Figlinolo, S.Naylor, J.Wang, and J.H.Langenheim, Phytochemistry, 1987, **26**, 3255.
15 G.P.Jones, B.P.Naidu, L.G.Paleg, E.R.T.Tiekink, and M.R.Snow, Phytochemistry, 1987, **26**, 3343.
16 H.Felbeck and S.Wiley, Biol.Bull.(Woods Hole, Mass.), 1987, **173**, 252.
17 R.Villalobos-Molina, J.P.Pardo, A.Saavedra-Molina, and E.Pina, Biochem. Cell. Biol., 1987, **65**, 1057.
18 J.Stenflo, A.Lundwall, and B.Dahlbaeck, Proc.Natl.Acad.Sci.U.S.A., 1987, **84**, 368.
19 J.Stenflo, A.K.Oehlin, W.G.Owen, and W.J.Schneider, J.Biol.Chem., 1988, **263**, 21.
20 S.C.Fry, J.Exp.Bot., 1987, **38**, 853.
21 H.E.Meyer and G.W.Mayr, Biol.Chem.(Hoppe Seyler's), 1987, **368**, 1607.
22 A.Moriguchi, Y.Hara, and S.Hatanaka, J.Antibiot., 1987, **40**, 904.
23 T.Ohta, S.Nakajima, I.Hatanaka, M.Yamamoto, Y.Shimmen, S.Nishimura, Z.Yamaizumi, and S.Nozoe, Phytochemistry, 1987, **26**, 565.
24 G.Kusano, H.Ogawa, A.Takahashi, S.Nozoe, and K.Yokoyama, Chem.Pharm.Bull., 1987, **35**, 3482.
25 D.Strack, D.Schmitt, H.Reznik, W.Boland, L.Grotjahn, and V.Wray, Phytochemistry, 1987, **26**, 2205.
26 G.N.Austin, P.D.Baird, H.F.Chow, L.E.Fellows, G.W.J.Fleet, R.J.Nash, J.M.Peach, R.J.Pryce, and C.H.Stirton, Tetrahedron, 1987, **43**, 1857.
27 Y.Kawahata, S.Takatsuto, N.Ikekawa, M.Murata, and S.Omura, Chem.Pharm.Bull., 1986, **34**, 3102.
28 S.Omura, M.Murata, N.Imamura, Y.Iwai, and H.Tanaka, J.Antibiot., 1984, **37**, 1324.
29 T.P.Holler, A.Spaltenstein, P.B.Hopkins, E.Turner, R.E.Klevit, and B.M.Shapiro, J.Org.Chem., 1987, **52**, 4420.
30 S.W.Elson, K.H.Baggaley, J.Gillett, S.Holland, N.H.Nicholson, J.T.Sime, and S.R.Woroniecki, J.Chem.Soc.,Chem.Commun., 1987, 1736; for synthesis of proclavanamic acid: K.H.Baggaley, J.T.Sime, N.H.Nicholson, S.W.Elson, J.Gillett, S.Holland, and S.R.Woroniecki, Ibid., p.1738.
31 S.P.F.Miller and J.Thompson, J.Biol.Chem., 1987, **262**, 16109.
32 M.Maeda, T.Kodama, T.Tanaka, H.Yoshizumi, T.Takemoto, K.Nomoto, and T.Fujita, Tetrahedron Lett., 1987, **28**, 633.
33 B.Starcher, G.Cook, P.M.Gallop, E.Hensen, and B.Shoulders, Connect.Tissue Res., 1987, **16**, 15.
34 G.R.Pettit, Y.Kamano, C.L.Herald, A.A.Tuinman, F.E.Boettner, H.Kizu, J.M.Schmidt, L.Baczynskj, K.B.Tomer, and R.J.Bontems, J.Am.Chem.Soc., 1987, **109**, 6883.
35 T.Teshima, M.Nishikawa, I.Kubota, T.Shiba, and S.Omura, Peptide Chemistry 1986, Proceedings of the 24th Symposium on Peptide Chemistry, Peptide Institute, Protein Research Foundation, Osaka, 1986, p.169 (Chem.Abs., 1988, **108**, 3113; 1986, **104**, 55380).
36 A.Tsugita, T.Uchida, H.W.Mewes, and T.Akata, J.Biochem. (Tokyo), 1987, **102**, 1593.
37 J.Taillades, J.Brugidou, R.Pascal, R.Sola, L.Mion, and A.Commeyras, Actual. Chim., 1986, 13
38 Y.Ohfune and N.Kurokawa, Yuki Gosei Kagaku Kyokaishi, 1986, **44**, 647.
39 See for example G.C.Barrett, in "Chemistry and Biochemistry of the Amino Acids", ed. G.C.Barrett, Chapman & Hall, London, 1985, p.276.
40 I.Ojima, J.Mol.Catal., 1986, **37**, 25; M.Tanaka and T.Kobayashi, Kagaku Kogyo Shiryo (Tsukuba-gun, Jpn), 1986, **21**, 35; A.M.Ajami and S.S.Yuan, "Synthesis and Applications of Isotopically-labelled Compounds", Ed.R.R.Muccino, Elsevier, Amsterdam, 1986, p.201.
41 C.Herdeis and R.Gebhard, Heterocycles, 1986, **234**, 1019.
42 J.Taillades, A.Rousset, M.Lasperas and A.Commeyras, Bull.Soc.Chim.Fr., 1986, 650.
43 M.Piovano, M.I.Gauido, P.Fiedler, and J.A.Garbarino, Rev.Latinoam.Quim.,

1987, **18**, 66.
44 P.N.Rao, J.E.Burdett, J.W.Cessac, C.M.DiNunno, D.M.Peterson, and H.K.Kim, Int.J.Pept.Prot.Res., 1987, **29**, 118.
45 H.Hilpert, Helv.Chim.Acta, 1987, **70**, 1307.
46 P.Münster and W.Steglich, Synthesis, 1987, 223.
47 J.R.Grierson and M.J.Adam, J.Labelled Compd.Radiopharm., 1986, **23**, 1019.
48 B.D.Gray and P.W.Jeffs, J.Chem.Soc.,Chem.Commun., 1987, 1329.
49 D.Dauzonne and R.Royer, Synthesis, 1987, 399.
50 S.Fujii, H.Kimoto, T.Okano, H.Muramatsu, and T.Ueda, Nagoya Kogyo Gijutsu Shikenso Hokuku, 1986, **35**, 228.
51 T.Mukaiyama, H.Suzuki, and T.Yamada, Chem.Lett., 1986, 915.
52 M.Mitani, O.Tachizawa, H.Takeuchi, and K.Koyama, Chem.Lett., 1987, 1029.
53 H.J.Zeiss, Tetrahedron Lett., 1987, **28**, 1255.
54 U.Schöllkopf, D.Pettig, U.Busse, E.Egert, and M.Dyrbusch, Synthesis, 1986, 737.
55 U.Schöllkopf, W.Kuehnle, E.Egert, and M.Dyrbusch, Angew.Chem., 1987, **99**, 480.
56 U.Schöllkopf and J.Bardenhagen, Liebigs Ann.Chem., 1987, 393.
57 R.Gull and U.Scholl kopf, Synthesis., 1985, 1052.
58 U.Schöllkopf, R.Hinrichs, and R.Lonsky, Angew.Chem., 1987, **99**, 137.
59 J.Bajgrowicz, A.El Achquar, M.L.Roumestant, C.Pigiere, and P.Viallefont, Heterocycles, 1986, **24**, 2165.
60 U.Schöllkopf, R.Tölle, E.Egert, and M.Nieger, Liebigs Ann.Chem., 1987, 399.
61 I.Ojima and H.-J.C.Chen, J.Chem.Soc.,Chem.Commun., 1987, 625.
62 Yu.N.Belokon, A.G.Bulychev, M.G.Ryzhov, S.V.Vitt, A.S.Batsanov, Yu.T.Struchkov, V.I.Bakhmutov, and V.M.Belikov, J.Chem.Soc.,Perkin Trans.I, 1986, 1865; Yu.N.Belokon, N.I.Chemoglazova, N.S.Garbalinskaya, M.B.Saporovskaya, K.A.Kochetkov, and V.M.Belikov, Izv.Akad.Nauk S.S.S.R., Ser.Khim., 1986, 2340.
63 P.Duhamel, J.J.Eddine, and J.-Y.Valnot, Tetrahedron Lett., 1987, **28**, 3801.
64 K.Suzuki, Y.Hirami, M.Taniai, T.Mohri, C.Goda, R.Fujiyama, and S.Kiyoota, Nippon Kagaku Zasshi, 1987, 186.
65 H.Kunz and W.Sager, Angew.Chem., 1987, **99**, 595.
66 L.D.Arnold, J.C.G.Drover, and J.C.Vederas, J.Am.Chem.Soc., 1987, **109**, 4649.
67 E.I.Karpeskaya, E.S.Levitina, L.F.Godunova, and E.I.Klabunovskii, J.Mol. Catal., 1986, **34**, 129.
68 D.S.Matteson and E.C.Beedle, Tetrahedron Lett., 1987, **28**, 4499.
69 A.Durealt, I.Tranchepain, C.Greck, and J.-C.Depezay, Tetrahedron Lett., 1987, **28**, 3341.
70 D.Seebach, E.Juaristi, D.D.Miller, C.Schickli, and T.Weber, Helv.Chim.Acta, 1987, **70**, 237.
71 D.A.Evans, E.B.Sjögren, A.E.Weber, and R.Elom, Tetrahedron Lett., 1987, **28**, 39.
72 D.A.Evans and A.E.Weber, J.Am.Chem.Soc., 1987, **109**, 7151.
73 D.A.Evans, J.A.Ellman, and R.L.Dorow, Tetrahedron Lett., 1987, **28**, 1123.
74 B.D.Zwick, A.M.Arif, A.T.Patton, and J.A.Gladysz, Angew.Chem., 1987, **99**, 921.
75 A.Karim, A.Mortreux, and F.Petit, J.Organomet.Chem., 1986, **312**, 375; C.R.Landis and J.Halpern, J.Am.Chem.Soc., 1987, **109**, 1746; H.Brunner, A.Knott, M.Kunz, and E.Thalhammer, J.Organomet.Chem., 1986, **308**, 55; W.Vocke, R.Haenel, and F.H.Flöther, Chem.Tech.(Leipzig), 1987, **39**, 123.
76 E.I.Klabunovskii, E.S.Levitina, L.N.Kaigorodova, D.D.Gogoladze, L.F.Godunova, E.I.Karpeiskaya, and G.O.Chivadze, Izv.Akad.Nauk S.S.S.R., Ser.Khim., 1987, 849; Zh.Org.Khim., 1987, **23**, 343.
77 Sh.G.Bitiev, G.V.Cheltsova, E.I.Karpeiskaya, and E.I.Klabunovskii, Izv.Akad.Nauk S.S.S.R., Ser.Khim., 1986, 2479.
78 J.E.Baldwin, R.L.Dyer, S.C.Ng, A.J.Pratt, and M.A.Russell, Tetrahedron Lett., 1987, **28**, 3743.
79 F.Bjorkling, J.Boutelje, M.Hjalmarsson, K.Hult, and T.Norin, J.Chem.Soc., Chem.Commun., 1987, 1041.
80 N.Makiguchi, Kagaku Kogyo, 1987, **38**, 439; D.M.Anderson, in "Biotechnology", Eds. C.P.Hohenberg and H.Sahm, Fischer, Stuttgart, 1987, Vol.1, p.41; K.Soda and H.Tanaka, Ibid., p.93; H.Enei, Hakko to Kogyo, 1986, **44**, 1070.
81 D.J.Rozzell, Methods Enzymol., 1987, **136**, 479.
82 K.Yokozeki, K.Sano, C.Eguchi, H.Iogami, and K.Mitsugi, Agric.Biol.Chem.,

1987, **51**, 729; K.Yokozeki, K.Sano, C.Eguchi, K.Yamada, and K.Mitsugi, Idem, p.363.
83 K.Yokozeki, S.Nakamori, C.Eguchi, K.Yamada, and K.Mitsugi, Agric.Biol.Chem., 1987, **51**, 355; K.Yokozeki, S.Nakamori, S.Yamanaka, C.Eguchi, K.Mitsugi, and F.Yoshinaga, Idem, p.713; K.Yokozeki and K.Kubota, Idem, p.721.
84 E.Schmidt, D.Vasic-Racki, and C.Wandrey, Appl.Microbiol.Biotechnol., 1987, **26**, 42.
85 K.Yokozeki, E.Majima, K.Izawa, and K.Kubota, Agric.Biol.Chem., 1987, **51**, 963.
86 M.Soukup, B.Wipf, E.Hochuli, and H.G.W.Leuenberger, Helv.Chim.Acta, 1987, **70**, 232.
87 K.M.Lee and R.W.Woodard, Misaengmul Kwa Sanop, 1987, **13**, 10.
88 Y.Kokusenya, S.Nakajima, and M.Matsuoka, Denki Kagaku Oyubi Kogyo Butsuri Kagaku, 1987, **55**, 235.
89 K.Tanaka, S.Yoshifuji, and Y.Nitta, Chem.Pharm.Bull., 1986, **34**, 3879.
90 A.J.Ozinskas and G.A.Rosenthal, Bioorg.Chem., 1986, **14**, 157.
91 H.Ripperger, J.Prakt.Chem., 1986, **328**, 719.
92 J.E.Baldwin, M.Otsuka, and P.M.Wallace, Tetrahedron, 1986, **42**, 3097.
93 B.P.Bashyal, H.F.Chow, L.E.Fellows, and G.W.J.Fleet, Tetrahedron, 1987, **43**, 415.
94 K.Hashimoto, K.Konno, H.Shirahama, and T.Matsumoto, Chem.Lett., 1986, 1399.
95 S.Takano, Y.Iwabuchi, and K.Ogasawara, J.Am.Chem.Soc., 1987, **109**, 5523.
96 J.Cooper, D.W.Knight, and P.T.Gallagher, J.Chem.Soc.,Chem.Commun., 1987, 1220.
97 J.E.Baldwin and C.-S.Li, J.Chem.Soc.,Chem.Commun., 1987, 166.
98 S.J.B.Tendler, M.D.Threadgill, and M.J.Tisdale, J.Chem.Soc.,Perkin Trans.I, 1987, 2617.
99 S.Wu, C.Zhou, and Y.Jiang, Synth.Colmmun., 1986, **16**, 1479.
100 A.Fadel and J.Salaun, Tetrahedron Lett., 1987, **28**, 2243.
101 Y.Jiang, Y.Ma, Z.Chen, and G.Li, Yingyong Huaxue, 1987, **4**, 44 (Chem.Abs., 1987, **107**, 198875).
102 M.Paventi and J.T.Edward, Can.J.Chem., 1987, **65**, 282.
103 M.Suzuki, G.F.Orr, and C.H.Stammer, Bioorg.Chem., 1987, **15**, 43.
104 D.Obrecht and H.Heimgartner, Helv.Chim.Acta, 1987, **70**, 102, 329.
105 G.Cipens, V.A.Slavinskaya, D.Sile, D.Kreile, A.Krikis, A.Strautina, and A.Gutmanis, Latv.P.S.R.Zinat.Akad.Vestis,Kim.Ser., 1986, 647.
106 M.Takasaki and K.Harada, Chem.Lett.,1987, 365.
107 D.W.McPherson, K.Rahman, I.Martinez, and P.B.Shevlin, Origins Life Evol. Biosphere, 1987, **17**, 275.
108 N.P.Kuzimina, Yu.A.Matveets, and E.V.Khoroshilova, Khim.Fiz., 1986, **5**, 1168.
109 J.Onoe and T.Kawai, J.Chem.Soc.,Chem.Commun., 1987, 1480.
110 C.Shen, L.Yang, S.L.Miller, and J.Oro, Origins Life Evol.Biosphere, 1987, **17**, 295.
111 J.H.McClendon, Origins Life Evol.Biosphere, 1987, **17**, 401.
112 S.L.Miller, Chem.Scr., 1986, **26B**, 5; A.Brack, Adv.Space Res., 1986, **6**, 17; Idem., Origins Life Evol.Biosphere, 1987, **17**, 367.
113 J.Pospisek and K.Blaha, Collect.Czech.Chem.Commun., 1987, **52**, 514.
114 M.P.Bueno, C.Cativiela, C.Fimol, J.A.Mayoral, and C.Jaime, Can.J.Chem., 1987, **65**, 2182.
115 N.Sakura, K.Hirose, and T.Hashimoto, Chem.Pharm.Bull., 1986, **34**, 3506.
116 H.L.Sham, H.Stein, and J.Cohen, J.Chem.Soc.,Chem.Commun., 1987, 1792.
117 H.L.Sham, C.A.Rempel, H.Stein, and J.Cohen, J.Chem.Soc., Chem.Commun., 1987, 683.
118 M.Thaniung and L.G.Wistrand, Helv.Chim.Acta, 1986, **69**, 1711.
119 Y.Vo-Quang, D.Marais, L.Vo-Quang, F.Le Goffic, A.Thiery, M.Maestracci, A.Arnaud, and P.Galzy, Tetrahedron Lett., 1987, **28**, 4057.
120 K.Kawada and T.Tsushima, Kagaku Kogyo, 1987, **38**, 164.
121 M.Akhtar, M.A.Cohen, and D.Gani, Tetrahedron Lett., 1987, **28**, 2413.
122 K.H.Hsieh, P.Needleman, and G.R.Marshall, J.Med.Chem., 1987, **30**, 1097.
123 S.Fujii, H.Kimoto, T.Okano, H.Muramatsu, and T.Ueda, Nagoya Kogyo Gijitsu Shikenso Hokoku, 1986, **35**, 107 (Chem.Abs., 1987, **106**, 176831).

124 Y.Kokusenya and M.Matsuoka, Denki Kagaku Oyobi Kogyo Butsuri Kagaku, 1987, 55, 174 (Chem.Abs., 1987, **107**, 134645).
125 Y.Kokusenya and M.Matsuoka, Denki Kagaku Oyobi Kogyo Butsuri Kagaku, 1987, 55, 257.
126 H.Kuzuhara, N.Watanabe, and M.Ando, J.Chem.Soc.,Chem.Commun., 1987, 95.
127 J.Wityak, S.J.Gould, S.J.Hein, and D.A.Keszler, J.Org.Chem., 1987, **52**, 2179.
128 M.Hasan, Stud.Org.Chem.(Amsterdam), 1986, **26**, 123.,
129 J.A.J.M.Vekemans, R.G.M.De Bruyn, R.C.H.M.Caris, A.J.P.M.Kokx, J.J.H.G. Konings, E.F.Godefroi, and G.J.F.Chittenden, J.Org.Chem., 1987, **52**, 1093.
130 K.Clinch, A.Vasella, and R.Schauer, Tetrahedron Lett., 1987, **28**, 6425.
131 B.P.Bashyal, H.F.Chow, and G.W.J.Fleet, Tetrahedron, 1987, **43**, 423; see also ref.93.
132 N.Passerat and J.Bolte, Tetrahedron Lett., 1987, **28**, 1277.
133 C.Shin, Y.Yonezawa, and T.Obara, Heterocycles, 1986, **24**, 1561.
134 C.Shin, T.Obara, S.Segami, and Y.Yonezawa, Tetrahedron Lett., 1987, **28**, 3827.
135 T.Weber, R.Aeschimann, T.Maetzke, and D.Seebach, Helv.Chim.Acta, 1986, **69**, 1365.
136 N.A.Thornberry, H.G.Bull, D.Taub, W.J.Greenlee, A.A.Patchett, and E.H.Cordes, J.Am.Chem.Soc., 1987, **109**, 7543.
137 R.G.Shea, J.N.Fitzner, J.E.Fankhauser, A.Spattenstein, P.A.Carpino, R.M.Peevey, D.V.Pratt, B.J.Tenge,and P.B.Hopkins, J.Org.Chem., 1986, **51**, 5243.
138 S.Bory, M.Gaudry, and A.Marquet, Nouv.J.Chem., 1986, **10**, 709.
139 J.E.Baldwin, R.M.Adlington, and N.G.Robinson, J.Chem.Soc.,Chem.Commun., 1987, 153.
140 A.Evidente, G.Marino, G.Randazzo, G.Sannia, C.Bertucci, and C.Rosini, Chem.Scr., 1986, **26**, 359.
141 D.G.Melillo, R.D.Larsen, D.J.Mathre, W.F.Shukis, A.W.Wood, and J.R.Colleluori, J.Org.Chem., 1987, **52**, 5143.
142 O.Kanamitsu, N.Kitajima, and I.Nagoya, J.Ferment.Technol., 1987, **65**, 395.
143 U.Madsen, K.Schaumburg, L.Brehm, D.R.Curtis, and P.Krogsgaard-Larsen, Acta Chem.Scand.,Ser.B, 1986, **B40**, 92.
144 J.C.Hodges, Synthesis., 1987, 20
145 G.Goto, K.Kawakita, T.Okutani, and T.Miki, Chem.Pharm.Bull., 1986, **34**, 3202.
146 T.Kolasa, S.Sharma, and M.J.Miller, Tetrahedron Lett., 1987, **28**, 4973.
147 T.Kolasa and M.J.Miller, J.Org.Chem., 1987, **52**, 4978.
148 R.W.Feenstra, E.H.M.Stokkingreef, R.J.F.Nivard, and H.C.J.Ottenheijm, Tetrahedron Lett., 1987, **28**, 1215.
149 S.Murahashi and T.Shiota, Tetrahedron Lett., 1987, **28**, 6469.
150 M.J.Milewska and A.Chimiak, Tetrahedron Lett., 1987, **28**, 1817.
151 J.Viret, J.Gabard, and A.Collet, Tetrahedron, 1987, **43**, 891.
152 W.Oppolzer and R.Moretti, Helv.Chim.Acta, 1986, **69**, 1923.
153 A.E.Miller and J.J.Bischoff, Synthesis, 1986, 777.
154 T.Hertzberg, T.Kuehler, and M.Nilsson, Acta Chem.Scand.,Ser B, 1986, **B40**, 387.
155 A.Oku, N.Horiie, and T.Harada, Bull.Chem.Soc.Jpn., 1987, **60**, 609.
156 H.T.Nagasawa, J.A.Elberling, and J.C.Roberts, J.Med.Chem., 1987, **30**, 1373.
157 J.Matos, F.M.Raushel, and C.H.Wong, Biotechnol.Appl.Biochem., 1987, **9**, 39.
158 H.J.Reich and C.P.Jasperse, J.Am.Chem.Soc., 1987, **109**, 5549.
159 D.H.R.Barton, D.Bridon, Y.Herve, P.Potier, J.Thierry, and S.Z.Zard, Tetrahedron, 1986, **42**, 4983.
160 A.D.Mikityuk, Yu.A.Strepikheev, B.A.Kashemirov, and P.S.Khokhov, Zh.Obshch. Khim., 1987, **57**, 305.
161 D.H.Kindler and M.M.Ames, J.Org.Chem., 1987, **52**, 2452.
162 P.Serafinowskowski, Nucleic Acids Res., 1987, **15**, 1121.
163 H.Yamada, K.Kuruyama, T.Eguchi, and M.Kajiwara, J.Labelled Compd. Radiopharm., 1987, **24**, 561.
164 P.J.Reider, R.S.E.Conn, P.Davis, V.J.Grenda, A.J.Zambito, and E.J.J.Grabowski, J.Org.Chem., 1987, **52**, 3326.
165 A.Haedener and C.Tamm, J.Labelled Compd.Radiopharm., 1987, **24**, 1291.
166 Q.Shen and H.Li, Hejishu, 1987, **10**, 48.

167 J.P.Beaucourt, J.Labelled Compd.Radiopharm., 1986, **23**, 553.
168 D.Ego, J.P.Beaucourt, and L.Pichat, J.Labelled Compd.Radiopharm., 1986, **23**, 229.
169 P.Johnstroem, S.Stone-Elander, K.Ericson, M.Mosskin, and M.Bergstroem, Appl.Radiat.Isot., 1987, **38**, 729.
170 C.Halldin, K.O.Schoeps, S.Stone-Elander, and F.A.Wiesel, Eur.J.Nucl.Med., 1987, **13**, 288.
171 R.Iwata, T.Ido, T.Takahashi, H.Nakanishi, and S.Iida, Appl.Radiat.Isot., 1987, **38**, 97.
172 J.R.Ropchan, A.R.Ricci, G.C.Low, M.E.Phelps, and J.R.Barrio, Appl.Radiat.Isot., 1986, **37**, 1013.
173 J.M.Bolster, W.Vaalburg, P.H.Elsinga, H.Wynberg, and M.G.Woldring, Appl.Radiat.Isot., 1986, **37**, 1069.
174 G.Antoni and B.Laengstroem, Acta Chem.Scand.,Ser.B, 1986, **B40**, 152.
175 G.Antoni and B.Laengstroem, Appl.Radiat.Isot., 1987, **38**, 655.
176 C.Halldin and B.Laengstroem, J.Labelled Compd.Radiopharm., 1986, **23**, 715.
177 J.E.Baldwin, S.C.Ng, A.J.Pratt, M.A.Russell, and R.L.Dyer, Tetrahedron Lett., 1987, **28**, 2303.
178 T.E.Walker and R.E.London, Appl.Environ.Microbiol., 1987, **53**, 92.
179 B.R.Branchini, F.G.Prendergast, G.A.Spencer, J.D.Hugdahl, B.D.Ray, and M.D.Kemple, J.Labelled Compd.Radiopharm., 1987, **24**, 637.
180 N.D.Ledford, C.R.Cutcliff, H.B.Wood, and M.Barfield, Org.Prep.Proced.Int., 1987, **19**, 209.
181 H.Parnes, E.J.Shelton, and G.T.Huang, Int.J.Pept.Prot.Res., 1986, **28**, 403.
182 Z.Yan, D.Feng, Y.Chen, and H.Sheng, Youji Huaxue, 1987, 216 (Chem.Abs., 1987, **108**, 22234).
183 T.Elbert, Isotopy, 1985, **26**, 153 (Chem.Abs., 1987, **107**, 198863).
184 R.H.D.Lambrecht, G.Slegers, G.Mannens, and A.Claeys, Enzyme Microb.Technol., 1987, **9**, 221.
185 E.Presecan, A.Ivanof, A.Mocanu, N.Palibroda, M.Bologa, V.Gorun, M.Oarga, and O,Barzu, Enzyme Microb.Technol., 1987, **9**, 663.
186 T.Furuta, Y.Kasuya, H.Takahashi, and S.Baba, J.Chem.Res.,Synop., 1987, 86.
187 T.Chaly and M.Diksic, J.Nucl.Med., 1986, **27**, 1896.
188 M.J.Adam, T.J.Ruth, J.R.Grierson, B.Abeysekera, and B.D.Palc, J.Nucl.Med., 1986, **27**, 1462.
189 P.H.Stern and R.M.Hoffman, Anal.Biochem., 1986, **158**, 408.
190 S.Chen, S.Bai, X.He, F.Wang, and Y.Ma, Yuanzineng Kexue Jishu, 1986, **20**, 136.
191 A.Plenevaux, R.Cantineau, M.Guillaume, L.Christaens, and G.Tihange, Appl.Radiat.Isot., 1987, **38**, 59.
192 J.D.Weinstein, S.M.Mayer, and S.I.Beale, Plant Physiol., 1987, **84**, 244.
193 A.Kah and D.Doernemann, Z.Naturforsch.,C: Biosci., 1987, **42**, 209.
194 A.Dureault, I.Tranchepain, and J.-C.Depezay, Synthesis, 1987, 491.
195 J.P.Wolf and H.Pfander, Helv.Chim.Acta, 1987, **70**, 116.
196 S.Takano, M.Yanase, Y.Sekiguchi, and K.Ogasawara, Tetrahedron Lett., 1987, **28**, 1783.
197 K.Barlos, P.Mamos, D.Papaioannou, and S.Patrianakou, J.Chem.Soc.,Chem.Commun., 1987, 1583.
198 D.Harris, K.L.Bhat, and M.M.Jouillie, Tetrahedron Lett., 1987, **28**, 2837.
199 R.G.Andrew, R.E.Conrow, J.D.Elliott, W.S.Johnson, and S.Ramezani, Tetrahedron Lett., 1987, **28**, 6535.
200 H.Kogen and T.Nishi, J.Chem.Soc.,Chem.Commun., 1987, 311.
201 M.Sakaitani and Y.Ohfune, Tetrahedron Lett., 1987, **28**, 3987.
202 P.Jouin, B.Castro, and D.Nisato, J.Chem.Soc.,Perkin Trans.I, 1987, 1177.
203 J.D.Aebi, M.K.Dhaon, and D.H.Rich, J.Org.Chem., 1987, **52**, 2881.
204 D.A.Evans and A.E.Weber, J.Am.Chem.Soc., 1986, **108**, 6757.
205 U.Schmidt and W.Siegel, Tetrahedron Lett., 1987, **28**, 2849.
206 R.D.Tung and D.H.Rich, Tetrahedron Lett., 1987, **28**, 1139.
207 N.Yamasaki, M.Murakami, and T.Mukaiyama, Chem.Lett., 1986, 1013.
208 J.M.Brown, A.P.James, and L.M.Prior, Tetrahedron Lett., 1987, **28**, 2179.
209 M.Seki, H.Kubota, T.Moriya, M.Yamagishi, S.Nishimoto, and K.Matsumoto, Chem.Pharm.Bull., 1986, **34**, 4516.
210 D.Seebach and H.Esterman, Tetrahedron Lett., 1987, **28**, 3103.

211 U.R.Joshi and P.A.Limaye, Indian J.Chem.,Sect.B, 1986, **25B**, 1176.
212 S.M.Brandstadter, I.Ojima, and K.Hirai, Tetrahedron Lett., 1987, **28**, 613.
213 R.Yoshioka, M.Tohyama, O.Ohtsuki, S.Yamada, and I.Chibata, Bull.Chem.Soc. Jpn., 1987. **60**, 649.
214 W.H.Pirkle and P.S.Reno, J.Am.Chem.Soc., 1987, **109**, 7189.
215 T.Shiraiwa, H.Miyazaki, T.Imai, M.Sunami, and H.Kurokawa, Bull.Chem.Soc. Jpn., 1987, **60**, 661.
216 T.Shiraiwa, H.Miyazaki, A.Uramoto, M.Sunami, and H.Kurokawa, Bull.Soc.Chem. Jpn., 1987, **60**, 1465.
217 T.Shiraiwa, H.Miyazaki, Y.Sakamoto, and H.Kurokawa, Bull.Chem.Soc.Jpn., 1986, **59**, 2331.
218 T.Shiraiwa, H.Yoshida, K.Mashima, and H.Kurokawa, Nippon Kagaku Kaishi, 1986, **6**, 177.
219 T.Shiraiwa, Y.Sado, M.Komure, and H.Kurokawa, Chem.Lett., 1987, 621.
220 T.Fukuhara, M.Isoyama, A.Shimada, M.Itoh, and S.Yuasa, J.Chromatogr., 1987, **387**, 562.
221 E.Alvira, L.Vega, and C.Girardet, Chem.Phys., 1987, **118**, 233.
222 F.Tsvetkov and U.Mingelgrin, Clays Clay Miner., 1987, **35**, 391.
223 Y.Dobashi and S.Hara, J.Org.Chem., 1987, **52**, 2490.
224 W.F.Lindner and I.Hirschboek, J.Liq.Chromatogr., 1986, **9**, 551.
225 W.H.Pirkle and T.C.Pochapsky, J.Am.Chem.Soc., 1987, **109**, 5975.
226 W.H.Pirkle, T.C.Pochapsky, G.S.Mahler, D.E.Corey, D.S.Reno, and D.M.Alessi, J.Org.Chem., 1986, **51**, 4991.
227 N.Oi and H.Kitahara, J.Liq.Chromatogr., 1986, **9**, 511.
228 I.Cho, K.M.Whang, and J.G.Park, J.Polym.Sci.,Part C:Polym.Lett., 1987, **25**, 19.
229 M.T.Aubel and L.B.Rogers, J.Chromatogr., 1987, **408**, 99.
230 K.Ravichandran and L.B.Rogers, J.Chromatogr., 1987, **402**, 49.
231 T.Shinbo, T.Yamaguchi, K.Nishimura, and M.Segiura, J.Chromatogr., 1987, **405**, 145.
232 Y.Yoichi, K.Saigo, K.Tachibana, and M.Hasegawa, Chem.Lett., 1986, 1347.
233 R.Chenevert and M.Letourneau, Chem.Lett., 1986, 1151.
234 B.I.Glaenzev, K.Faber, and H.Griengl, Tetrahedron, 1987, **43**, 771.,
235 I.Minematsu, Y.Shimohigashi, M.Waki, and N.Izumiya, Mem.Fac.Sci.,Kyushi Univ.,Ser.C, 1986, **15**, 269 (Chem.Abs., 1987, **107**, 7545).
236 S.F.Mason, Biochem.Pharmacol., 1988, **37**, 1.
237 G.E.Tranter, Nachr.Chem.Tech.Lab., 1986, **34**, 866, 871, 874, 876.
238 D.K.Kondepudi, Biosystems, 1987, **20**, 75.
239 E.Conte, Nuovo Cimento Soc.Ital.Fis.D, 1987, **9D**, 497.
240 W.J.Meiring, Nature, 1987, **329**, 712.
241 E.Conte and M.Pieralice, Nuovo Cimento Soc.Ital.Fis.D, 1987, **9D**, 283.
242 F.C.Frank, Biochim.Biophys.Acta, 1953, **11**, 459.
243 V.Babovic, I.Gutman, and S.Jokic, Z.Naturforsch.A. Phys.Sci., 1987, **42**, 1024.
244 E.Paparinasam, S.Natarajan, and N.C.Shivaprakash, Int.J.Pept.Prot.Res., 1986, **28**, 525.
245 P.Toffoli, P.Khodadad, N.Rodier, and J.Astoin, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1987, **C43**, 945.
246 B.Chevrier, D.Moras, V.L.R.Jeannoda, E.E.Creppy, and G.Dirheimer, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1986, **C42**, 1632.
247 A.H.J.Wang and S.K.Burley, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1987, **C43**, 1011.
248 V.Nastopoulos, G.Germain, P.Cordopatis, and S.Voliotis, Acta Crystallogr., Sect.C: Cryst.Struct.Commun., 1987, **C43**, 375.
249 C.P.Huber, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1987, **C43**, 902.
250 S.K.Burley and A.H.J.Wang, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1987, **C43**, 797.
251 M.Coll, J.A.Subirama, X.Solans, M.Font-Altaba, and R.Mayer, Int.J.Pept. Prot.Res., 1987, **29**, 708.
252 V.Busetti, H.C.J.Ottenheijm, H.J.M.Zeegers, D.Ajo, and M.Casavin, Recl.Trav.Chim.Pays-Bas, 1987, **106**, 151.

253 P.Gordopatis, S.Voliotis, V.Nastopoulos, G.Germain, and P.Main, Acta Crystallogr., Sect.C: Cryst.Struct.Commun., 1987, **C43**, 344.
254 Y.Yokomori, D.W.Deerfield, R.G.Hiskey, and D.J.Hodgson, Acta Crystallogr., Sect.C: Cryst.Struct.Commun., 1987, **C43**, 1567.
255 S.Talluri, G.T.Montelione, G.Van Duyne, L.Piela, J.Clardy, and H.A.Scheraga, J.Am.Chem.Soc., 1987, **109**, 4473.
256 K.Kabato and Y.Sasaki, J.Chem.Soc.,Chem.Commun., 1987, 670.
257 F.Mitte, R.Mensinger, and R.Borsdorf, Z.Chem., 1986, **26**, 107.
258 N.Koda, S.Yamaguchi, and T.Mori, Tokyo Jikeikai Ika Daigaku Zasshi, 1987, **102**, 1263, 1269 (Chem.Abs., 1988, **108**, 2889).
259 E.Pederoso, A.Grandas, and M.Dolors-Ludevid, J.Heterocycl.Chem., 1986, **23**, 921.
260 V.I.Dubrovin, S.V.Tarabakin, and T.A.Smetanina, Khim.-Farm.Zh., 1987, **21**, 877.
261 M.S.Morales-Rios and P.Joseph-Nathan, J.Heterocycl.Chem., 1986, **23**, 1617.
262 C.A.McDowell, A.Naito, D.L.Sastry, and K.Takegoshi, J.Magn.Reson., 1986, **69**, 283.
263 B.V.R.R.K.Murty, G.K.Kishore, D.K.Rao, and G.Satanandam, Pramana, 1987, **28**, 45.
264 J.Dorie, J.P.Gouesnard, B.Mechin, and P.Mellet, J.Mol.Struct., 1986, **144**, 185.
265 D.Krois and H.Lerner, Monatsh.Chem., 1986, **117**, 1205.
266 S.Higuchi, K.Tanaka, and S.Tanaka, Nippon Kagaku Kaishi, 1986, 1632.
267 T.Wakamiya, T.Teshima, and T.Shiba, Iyo Masu Kenkyukai Koenshu, 1986, **11**, 35.
268 S.Okude, F.Matsushima, H.Kuze, and T.Shimizu, Jpn.J.Appl.Phys., Part I, 1987, **26**, 627.
269 D.Holtkamp, H.Kempken, P.Kluesener, and A.Benninghaven, Springer Proc.Phys., 1986, **9**, 62 (Chem.Abs., 1987, **107**, 97073).
270 G.R.Pettit, G.M.Cragg, C.W.Holzapfel, A.A.Tuinman, and D.P.Gieschen, Anal. Biochem., 1987, **162**, 236.
271 G.Bojesen, J.Am.Chem.Soc., 1987, **109**, 5557.
272 A.Kolbe and A.Kolbe, Z.Chem., 1987, **27**, 74.
273 C.I.Jose, A.A.Belhakar, and M.S.Agashe, Biopolymers, 1987, **26**, 1315.
274 H.Matsuura, K.Hasegawa, amd T.Miyazawa, Spectrochim.Acta, Part A, 1986, **42A**, 1181.
275 F.Lopez-Arbeloa, R.Goedewieck, F.Ruttens, F.C.De Schryver and M.Sisido, J.Am.Chem.Soc., 1987, **109**, 3068.
276 T.A.Hatton, A.C.S. Symp.Ser., 1987, **342**, 170.
277 M.Housana, Kemikaru Enjiniyaringu, 1986, **31**, 965 (Chem.Abs., 1987, **107**, 35805).
278 K.E.Joergensen and M.I.Sheikh, Biochem.J., 1987, **248**, 533.
279 S.Inokuma, T.Hayase, and T.Kuwamura, Yukagaku, 1987, **36**, 487 (Chem.Abs., 1988, **108**, 33666).
280 P.M.Hardy and A.E.Langran-Goldsmith, Int.J.Pept.Prot.Res., 1986, **28**, 230.
281 L.R.Aleksandrova, V.N.Andreev, V.A.Bogdanovskaya, and A.Yu.Safronov, Elektrokhimiya, 1987, **23**, 276 (Chem.Abs., 1987, **106**, 145926).
282 G.Horanyi and E.M.Rizmayer, Electrochim.Acta, 1987, **32**, 433 (Chem.Abs., 1987, **106**, 145941).
283 S.Miyagishi, S.Matsumura, T.Asakawa, and M.Nishida, Bull.Chem.Soc.Jpn., 1986, **59**, 557.
284 G.Barone, G.Castronuovo, V.Elia, and C.Giancola, J.Therm.Anal., 1985, **30**, 1367.
285 W.W.Wendlandt, Thermochim.Acta, 1987, **114**, 381.
286 J.D.Ewbank, V.J.Klimkowski, K.Siam, and L.Schafer, J.Mol.Struct., 1987, **160**, 275.
287 A.-M.Sapse, L.Mallah-Levy, S.B.Daniels, and B.W.Erickson, J.Am.Chem.Soc., 1987, **109**, 3526.
288 U.C.Singh, F.K.Brown, P.A.Bash, and P.A.Kollman, J.Am.Chem.Soc., 1987, **109**, 1607.
289 J.A.Sordo, M.Robst, G.Corongiu, S.Chin, and E.Clementi, J.Am.Chem.Soc., 1987, **109**, 1702.
290 R.W.L.Kimber and C.V.Griffin, Geochim.Cosmochim.Acta, 1987, **51**, 839.

291 G.A.Goodfriend, Quat.Res., 1987, **28**, 374.
292 A.Julg, R.Lafont, and G.Perinet, Quat.Sci.Rev., 1987, **6**, 25.
293 L.Van Meervelt, V.C.V.Tijd., 1986, **13**, 4 (Chem.Abs., 1987, **106**, 175320).
294 Yu.N.Belokon, V.I.Tavarov, T.F.Saveleva, M.B.Saporovskaya, and V.M.Belikov, Makromol.Chem., 1986, **187**, 1065.
295 I.A.Yamskov, T.V.Tikhonova, V.E.Tikhonov, and V.A.Davankov, Izv.Akad.Nauk S.S.S.R.,Ser.Khim., 1986, 1593.
296 X.Ding and Q.Jiang, Huaxue Shijie, 1986, **27**, 545 (Chem.Abs., 1987, **107**, 78220).
297 I.Tabushi, Y.Kuroda, and M.Yamada, Tetrahedron Lett., 1987, **28**, 5095.
298 W.D.Lubell and H.Rapoport, J.Am.Chem.Soc., 1987, **109**, 236.
299 L.A.Carpino, Acc.Chem.Res., 1987, **20**, 401.
300 R.C.de L.Milton, E.Becker, S.C.F.Milton, J.E.J.Baxter, and J.F.Elsworth, Int.J.Pept.Prot.Res., 1987, **30**, 431.
301 L.Kisfaludy and L.Otvos, Synthesis, 1987, 510.
302 W.J.Paleveda, F.W.Holly, and D.F.Veber, Org.Synth., 1985, **63**, 171.
303 P.I.Svirskaya, C.C.Leznoff, and M.Steinnan, J.Org.Chem., 1987, **52**, 1362.
304 E.Wuensch, W.Graf, O.Keller, W.Keller, and G.Wersin, Synthesis, 1986, 958.
305 J.Metzger and G.Jung, Liebigs Ann.Chem., 1987, 895.
306 S.Kirkeley, P.Jakobsen and D.Moe, Anal.Lett., 1987, **20**, 303.
307 W.Greenaway and F.R.Whatley, J.Chromatogr., 1987, **409**, 383.
308 H.O.Kim, R.K.Olsen, and O.S.Choi, J.Org.Chem., 1987, **52**, 4531.
309 T.Chiba, J.Sakaki, and C.Kaneko, Yakugaki Zasshi, 1986, **106**, 154.
310 T.Munegumi, M.Fujita, T.Maruyama, S.Shiono, M.Takasaki, and K.Harada, Bull.Chem.Soc.Jpn., 1987, **60**, 249.
311 H.Kunz and H.-G.Lerchen, Tetrahedron Lett., 1987, **28**, 1873.
312 A.Castro, E.Iglesias, J.R.Leis, J.V.Tato, F.Meijide, and M.E.Pena, J.Chem. Soc.,Perkin Trans.II, 1987, 651.
313 S.Ram and L.D.Spicer, Tetrahedron Lett., 1987, **28**, 515.
314 A.Aramendia, V.Borau, C.Jimenet, J.M.Marinas, and M.E.Sempere, Can.J.Chem., 1987, **65**, 2791.
315 A.Ballio, Gazz.Chim.Ital., 1986, **116**, 519.
316 M.Tamura, J.Jacyno, and C.H.Stammer, Tetrahedron Lett., 1986, **27**, 5435.
317 I.Ruser and D.Chochkova, Kim.Ind.(Sofia), 1986, **58**, 445.
318 J.H.Jones and M.E.Wood, Synth.Commun., 1986, **16**, 1515.
319 D.Cantacazune, F.Pascal, and C.Guerreiro, Tetrahedron, 1987, **43**, 1823.
320 D.Cantacazune and C.Guerreiro, Tetrahedron Lett., 1987, **28**, 5153.
321 S.Plaue and D.Heissler, Tetrahedron Lett., 1987, **28**, 1401.
322 A.A.Mazurov, S.V.Antonenko, and S.A.Andronati, Khim.Prir.Soedin., 1986, 224.
323 M.Jaonadi, J.Martinez, B.Castro, G.Barcelo, G.Sennyey, and J.-P.Senet, J.Org.Chem., 1987, **52**, 2364.
324 P.Jouin, B.Castro, C.Zeggaf, A.Pantaloni, J.-P.Senet, S.Lecoller, and G.Sennyey, Tetrahedron Lett., 1987, **28**, 1661, 1665.
325 F.M.F.Chen and N.L.Benoiton, Can.J.Chem., 1987, **65**, 619.
326 F.M.F.Chen and N.L.Benoiton, Can.J.Chem., 1987, **65**, 1228.
327 F.M.F.Chen, Y.Lee, R.Steinauer, and N.L.Benoiton, Can.J.Chem., 613.
328 J.Cabre Castellvi and A.Palomo Coll, Afinidad, 1986, **43**, 401.
329 W.Gruszecki, M.Gruszecka, and H.Bradaczek, Liebigs Ann.Chem., 1987, 483.
330 A.Ookawa and K.Soai, J.Chem.Soc., Perkin I, 1987, 1465.
331 P.Renaud and D.Seebach, Helv.Chim.Acta, 1986, **69**, 1704.
332 K.Kamiyama, Y.Urano, S.Kobayashi, and M.Ohno, Tetrahedron Lett., 1987, **28**, 3123.
333 R.H.Schlessinger and E.J.Iwanowicz, Tetrahedron Lett., 1987, **28**, 2083.
334 R.B.Bates, F.A.Fletcher, K.D.Janda, and W.A.Miller, J.Org.Chem., 1984, **49**, 3038.
335 M.Muneer, R.K.Tikare, P.V.Kamat, and M.V.George, Can.J.Chem., 1987, **65**, 1624.
336 N.Kuramoto, T.Saki, K.Kato, K.Nagai, and S.Hayama, J.Colloid Interface Sci., 1987, **15**, 595.
337 R.Ueoka, Y.Matsumoto, T.Yoshino, N.Watanabe, K.Omura, and Y.Murakami, Chem. Lett., 1986, 1743.
338 R.Andriamanampisoa, B.Boyer, G.Lamaty, and J.P.Roque, Tetrahedron, 1987, **43**, 77.
339 Y.Ihara, E.Nakanishi, M.Nango, and J.Koga, Bull.Chem.Soc.Jpn., 1986, **59**,

1901.
340 D.S.Mahadevappa and A.S.Puttaswamy, Indian J.Chem.Sect.A, 1987, **26A**, 33.
341 D.Laloo and M.K.Mahanti, Afinidad, 1987, **44**, 324.
342 M.S.Ramachandran and T.S.Vivekamandam, Bull.Chem.Soc.Jpn., 1987, **60**, 3397.
343 B.T.Gowda and B.S.Sherigara, Oxid.Commun., 1986, **9**, 165.
344 L.M.Benzing-Purdic and J.A.Ripmeister, J.Carbohydr.Chem., 1987, **6**, 87.
345 D.H.R.Barton, J.Guilhem, Y.Herve, P.Potier, and J.Thierry, Tetrahedron Lett., 1987, **28**, 1413.
346 M.F.Aly, H.Ardill, R.Grigg, S.Leong-Ling, S.Rajviroongit, and S.S.Surendrakumar, Tetrahedron Lett., 1987, **28**, 6077.
347 E.Vedejs, J.W.Grisson, and J.K.Preston, J.Org.Chem., 1987, **52**, 3487.
348 H.Ardill, R.Grigg, V.Sridharan, and J.Malone, J.Chem.Soc.,Chem.Commun., 1987, 1296.
349 O.Yebdri and F.Texier, J.Heterocycl.Chem., 1986, **23**, 809.
350 S.Kim, S.B.Chang, and P.H.Lee, Tetrahedron Lett., 1987, **28**, 2735.
351 V.A.Burgess and C.J.Easton, Tetrahedron Lett., 1987, **28**, 2747.
352 T.Okamoto, M.Shimada, and S.Oka, Chem.Lett., 1987, 817.
353 S.Yoshifuji, K.Tanaka, F.Kawals, and Y.Nitta, Chem.Pharm.Bull., 1986, **34**, 3873.
354 M.Lee and J.W.Lown, J.Org.Chem., 1987, **52**, 5717.
355 J.E.Baldwin, M.North, and A.Flinn, Tetrahedron Lett., 1987, **28**, 3167.
356 J.E.Baldwin and A.Flinn, Tetrahedron Lett., 1987, **28**, 3605.
357 R.Bhatt, P.B.Gandhi, S.C.Armeta, and H.C.Chowdhry, Pak.J.Sci.Ind.Res., 1987, **30**, 347.
358 B.L.Milic, S.M.Djilas, and D.S.Vlaovic, Analyst, 1987, **112**, 779.
359 R.Albert, J.Danklmeier, H.Hoenig, and H.Kandolf, Synthesis, 1987, 635.
360 S.-T.Chen and K.-T.Wang, Synthesis, 1987, 581; S.-T.Chen, K.-T.Wang, and C.-H.Wong, J.Chem.Soc.,Chem.Commun., 1986, 1514.
361 B.D.Harris, K.L.Bhat, and M.M.Jouillie, Synth.Commun., 1986, **16**, 1815.
362 W.Dorsch and F.Effenberger, Chem.Ber., 1987, **120**, 55.
363 L.Gurarii and E.T.Mukmenev, Zh.Obshch.Khim., 1986, **56**, 2649.
364 N.A.Sasaki, C.Hashimoto, and P.Potier, Tetrahedron Lett., 1987, **28**, 6061.
365 F.Effenberger and T.Weber, Angew.Chem., 1987, **99**, 146.
366 J.Kohn and R.Langer, J.Am.Chem.Soc., 1987, **109**, 817.
367 Z.B.Alfassi, Radiat.Phys.Chem., 1987, **29**, 405.
368 D.L.Boger and D.Yohannes, J.Org.Chem., 1987, **52**, 5283.
369 D.W.Hobbs and W.C.Still, Tetrahedron Lett., 1987, **28**, 2805.
370 S.Ranganathan, D.Ranganathan, S.Singh, and D.Bhattacharyya, J.Chem.Soc., Chem.Commun., 1987, 1887.
371 S.Ranganathan, D.Ranganathan, and D.Bhattacharyya, J.Chem.Soc.,Chem. Commun., 1987, 1085.
372 L.D.Keys and G.A.Hamilton, J.Am.Chem.Soc., 1987, **109**, 2156.
373 D.M.Law, Physiol.Plant., 1987, **70**, 626.
374 J.R.Matos and C.H.Wong, Bioorg.Chem., 1987, **15**, 71.
375 C.O.Meese, Arch.Pharm.(Weinheim), 1987, **320**, 473.
376 D.K.Liu and S.G.Chang, Can.J.Chem., 1987, **65**, 770.
377 R.H.White, J.Labelled Compd.Radiopharm., 1987, **24**, 323.
378 C.N.Hsaio, L.Liu, and M.J.Miller, J.Org.Chem., 1987, **52**, 2201.
379 S.A.Sheldon and T.Shibamoto, Agric.Biol.Chem., 1987, **51**, 2473.
380 H.J.Reich and C.P.Jasperse, J.Am.Chem.Soc., 1987, **109**, 5549.
381 K.Kaleem, F.Chertok, and S.Erhan, J.Biol.Phys., 1987, **15**, 63.
382 M.Ruiz-Gayo, F.Albericio, E.Pedroso, and E.Giralt, J.Chem.Soc.,Chem. Commun., 1986, 1501.
383 F.Albericio, A.Grandas, A.Porta, E.Pedroso, and E.Giralt, Synthesis, 1987, 271.
384 R.Ramage and J.Green, Tetrahedron Lett., 1987, **28**, 2287.
385 C.J.Chivikas and J.C.Hodges, J.Org.Chem., 1987, **52**, 3591.
386 H.J.Keller, K.Q.Do, M.Zollinger, K.H.Winterhalter, and M.Cuenod, Anal. Biochem., 1987, **166**, 431
387 M.Shimizu, J.Phys.Soc.Jpn., 1987, **56**, 43.
388 A.Andreoni, Med.,Biol.,Environ., 1987, **15**, 57.
389 H.Droste and T.Wieland, Liebigs Ann.Chem., 1987, 901.
390 J.D.Kanner and O.Fennema, J.Agric.Food Chem., 1987, **35**, 71.

391 M.A.Krogull and O.Fennema, J.Agric.Food Chem., 1987, **35**, 66.
392 J.R.Wagner, H.Ali, R.Langlois, N.Brasseur, and J.E.Van Lier, Photochem. Photobiol., 1987, **45**, 507.
393 E.Reddi, C.R.Lambert, G.Jori, and M.A.J.Rodgers, Photochem.Photobiol., 1987, **45**, 345.
394 J.Butler, E.J.Land, A.J.Swallow, and W.A.Prutz, J.Phys.Chem., 1987, **91**, 3113.
395 K.Goda, M.Hisaoka, and T.Ueda, Biochem.Int., 1987, **15**, 635.
396 N.L.Vekshin, Stud.Biophys., 1987, **118**, 173.
397 S.G.Berlotti, M.Del V.Bohorquez, J.J.Cosa, N.A.Garcia, and C.M.Previtali, Photochem.Photobiol., 1987, **46**, 331.
398 A.Nishimura, Okayama Igakkai Zasshi, 1986, **98**, 827 (Chem.Abs., 1987, **107**, 35786).
399 E.J.Land, A.Thompson, T.G.Truscott, K.V.Subbarao, and M.R.Chedekel, Photochem.Photobiol., 1986, **44**, 697.
400 A.L.Simonyan, G.E.Khachatryan, S.Sh.Tatikyan, and T.S.M.Arakyan, Biol.Zh. Arm., 1986, **39**, 555.
401 "Amino Acid Analysis by Gas Chromatography", Eds. R.W.Zumwalt, K.C.Kuo, and C.W.Gehrke, 1987.
402 M.Aparici, V.Bori, J.Gonzalez, E.Iturgoyen, V.Liceras, S.Matoses, J.Perez de los Lobos, Laboratorio (Granada,Spain), 1986, **482**, 111.
403 R.W.Zumwalt, J.S.Absheer, F.E.Kaiser, and C.W.Gehrke, J.Assoc.Off.Anal. Chem., 1987, **70**, 147.
404 C.W.Gehrke, K.C.T.Kuo, F.E.Kaiser, and R.W.Zumwalt, J.Assoc.Off.Anal.Chem., 1987, **70**, 160.
405 R.W.Zumwalt, J.Desgres, K.C.T.Kuo, J.E.Pautz, and C.W.Gehrke, J.Assoc.Off. Anal.Chem., 1987, **70**, 253.
406 T.Furuta, Y.Kasuya, H.Shibasaki, and S.Baba, J.Chromatogr., 1987, **413**, 1.
407 S.L.Mackenzie, J.Assoc.Off.Anal.Chem., 1987, **70**, 151.
408 T.Miyata, Y.Okano, J.Nagata-Tanoue, S.Ijima-Miyamura, H.Iwamura, K.Takahama, and T.Hitoshi, Anal.Biochem., 1987, **163**, 303.
409 R.J.Early, J.R.Thompson, G.W.Sedgwick, J.M.Kelly, and R.J.Christopherson, J.Chromatogr., 1987, **416**, 15.
410 S.P.Stabler, P.D.Marcell, E.R.Podell, and R.H.Allen, Anal.Biochem., 1987, **162**, 185.
411 H.J.Chaves das Neves amd A.M.P.Vasconcelos, J.Chromatogr., 1987, **392**, 249.
412 S.L.Mackenzie, D.Tenaschuk, and G.Fortier, J.Chromatogr., 1987, **387**, 241.
413 H.Brueckner, I.Bosch, T.Graser, and P.Fuerst, J.Chromatogr., 1987, **395**, 569.
414 J.Velisek, T.Davidek, J.Davidek, P.Brezina, and C.Curda, Sb.UVTIZ Potravin. Vedy, 1986, **4**, 161 (Chem.Abs., 1987, **106**, 115923).
415 C.R.Krishnamurti and A.L.Schaefer, Nutr.Rep.Int., 1987, **35**, 683.
416 D.Yu, H.T.Stuewe and W.A.Koenig, Angew.Chem., 1987, **99**, 362.
417 M.Jemal and A.I.Cohen, J.Chromatogr., 1987, **392**, 442.
418 E.Bayer, H.Frank, J.Gerhardt, and G.Nicholson, J.Assoc.Off.Anal.Chem., 1987, **70**, 234.
419 I.Abe, H.Tsujioka, and T.Waso, Chem.Express, 1986, **1**, 335.
420 G.Wang, L.Zhou, S.Chen, H.Luo, and J.Wang, Fenxi Huaxue, 1987, **15**, 783 (Chem.Abs., 1988, **108**, 55064).
421 M.K.Park, J.S.Kang, J.H.Ryn, J.H.Park, and D.W.Jeon, Yakhak Hoechi, 1986 **30**, 47 (Chem.Abs., 1987, **106**, 176826).
422 R.Wettengel, ZfI-Mitt., 1986, **115**, 191.
423 R.Wettengel, J.Poerschmann, R.Herzschuh, G.Mehlhorn, R.Kliche, and L.Liebetrau, Nahrung, 1987, **31**, 39.
424 D.Liu and J.WQang, Sepu, 1987. **5**, 105.
425 P.J.M.Van der Oetelaar, J.R.C.M.Van Beckhoven, and H.J.Hoenders, J.Chromatogr., 1987, **388**, 441.
426 Y.Takata, Y.Fujii, H.Takahashi, and S.Ganno, Hitachi Hyoron, 1987, **69**, 333 (Chem.Abs., 1987, **107**, 55024).
427 L.B.James, J.Chromatogr., 1987, **408**, 291.
428 L.B.James, J.Chromatogr., 1987, **391**, 269.
429 L.B.James, J.Chromatogr., 1987, **404**, 321.
430 L.B.James, J.Chromatogr., 1987, **403**, 271.
431 L.B.James, J.Chromatogr., 1987, **410**, 176.

432 J.Wong and D.A.Applegarth, Clin.Chem.(Winston Salem), 1987, **33**, 1302.
433 W.Naumann, H.Noetzold, and G.Kempe, Z.Chem., 1987, **27**, 262.
434 A.M.Marx, H.Kronberg, and V.Neuhoff, J.Chromatogr., 1987, **393**, 407.
435 S.D.Mitrinovic, B.D.Medakovic, N.Djordjevic, S.M.Dragovic, and R.Kreacic, Iugoslav.Physiol.Pharmacol.Acta, 1986, **22**, 13.
436 R.Kraft, A.Otto, H.J.Zoepfl, and G.Etzold, Biomed.Environ.Mass Spectrom., 1987, **14**, 1.
437 R.Bhushan and I.Ali, J.Liq.Chromatogr., 1986, **9**, 3479.
438 R.Bhushan and G.P.Reddy, Anal.Biochem., 1987, **162**, 427.
439 R.Bhushan and I.Ali, J.Chromatogr., 1987, **392**, 460.
440 S.V.Kulikov and M.A.Samartsev, Khim.Prir.Soedin., 1987, 613.
441 K.Hirschberg, H.Faust, and A.Balint, Isotopenpraxis, 1987, **23**, 153.
442 G.Szepesi, Z.Pap-Sziklay, Z.Bertha-Somodi, and Z.Vegh, J.Pharm.Biomed. Anal., 1986, **4**, 799; E.Tyihak, Ibid., 1987, **5**, 191.
443 A.N.Boguslavskii and V.E.Egert, Khim.-Farm.Zh., 1987, **21**, 234.
444 T.M.Nogina, I.P.Stognii, O.R.Gvozdyak, O.A.Nesterenko, and E.I.Kvasnikov, Mikrobiol.Zh.(Kiev), 1987, **49**, 98.
445 N.S.Yang, S.G.Platt, and P.Christou, Anal.Biochem., 1987, **160**, 342.
446 M.H.Joseph and C.A.Marsden, in "H.P.L.C. of Small Molecules", Ed. C.K.Lim, IRL Press, Oxford, 1986, p.13.
447 R.F.Goldsmith, in "High Performance Liquid Chromatography in the Clinical Laboratory, Ed. D.C.Sampson, Australian Association for Clinical Biochemistry, Sydney, 1986, p.113.
448 R.Causon, D.Davies, and M.Brown, Life Sci., 1987, **41**, 909.
449 S.Hara, A.Dobashi, K.Kinoshita, T.Hondo, M.Saito, and M.Senda, J.Chromatogr., 1986, **371**, 153.
450 C.Hansson, B.Kaagedal, and M.Kaellberg, J.Chromatogr., 1987, **420**, 146.
451 J.L.Venthey, M.A.Bagnoud, and W.Haerdi, J.Chromatogr., 1987, **393**, 51.
452 T.A.Walker and D.J.Pietrzyk, J.Liq.Chromatogr., 1987, **10**, 161.
453 T.Nakagawa, A.Shibukawa, A.Kaihara, and H.Tanaka, Nippon Kagaku Kaishi, 1986, 1002 (Chem.Abs., 1987, **107**, 7541).
454 S.Levin and E.Grushka, J.Chromatogr., 1987, **384**, 249.
455 J.P.Richie and C.A.Lang, Anal.Biochem., 1987, **163**, 9.
456 A.Ichimura, M.Nakatsuka, K.Ogura, and T.Kiragawa, Nippon Kagaku Kaishi, 1986, 987.
457 T.Iwamoto, M.Yoshiura, and K.Iriyama, Bunseki Kagaku, 1987, **36**, 98.
458 J.Haginaka and J.Wakai, J.Chromatogr., 1987, **396**, 297.
459 Y.Hashimoto, S.Yamagata, and T.Hayakawa, Anal.Biochem., 1987, **160**, 362.
460 H.Gjerde, P.T.Normann, and J.Moerland, Biomed.Biochim.Acta, 1987, **46**, 53.
461 A.Csiba, L.Trezl, I.Rusznak,and T.Szarvas, Symp.Biol.Hung., 1986, **34**, 57.
462 M.J.Campello, E.Cortes, M.S.Aguilar, I.Ballester, and M.Moya, Tec.Lab., 1987, **11**, 21.
463 N.T.Soskel, Anal.Biochem., 1987, **160**, 98.
464 C.G.Zarkadas, J.A.Rochemont, G.C.Zarkadas, C.N.Karatzas, and A.D.Khalili, Anal.Biochem., 1987, **160**, 251.
465 Y.Yamaguchi, J.Haginaka, M.Kunitomo, H.Yasuda, and Y.Bando, J.Chromatogr., 1987, **422**, 53.
466 Y.Seyama, H.Inoue, M.Iwama, H.Watanabe, M.Irie, H.Orimo, and S.Yamashita, Rinsho Kagaku (Nippon Rinsho Kagakkai), 1987, **16**, 38 (Chem.Abs., 1988, **108**, 2830).
467 W.H.Mennicke, T.Kral, G.Krumbiegel, and N.Rittmann, J.Chromatogr., 1987, **414**, 19.
468 J.C.Rutledge and J.Riudy, Am.J.Chim.Pathol., 1987, **87**, 614.
469 J.T.Tyopponen, J.Chromatogr., 1987, **413**, 25.
470 A.Negro, S.Garbisa, L.Gotte, and M.Spina, Anal.Biochem., 1987, **160**, 39.
471 L.P.Pang, K.C.Ho, M.G.Jones, and C.K.Cheung, J.Chromatogr., 1987, **386**, 309.
472 G.J.Hughes, S.Frutiger, and C.Fonck, J.Chromatogr., 1987, **389**, 327.
473 E.Andersson. E.Haakanson, J.Larsson, and J.Maartensson, J.Chromatogr., 1987, **414**, 174.
474 P.E.Minkler, S.T.Ingalls, R.L.Griffin, and C.L.Hoppel, J.Chromatogr., 1987, **413**, 33.
475 D.F.Bushey, D.M.Law, and P.J.Davies, Anal.Biochem., 1987, **167**, 31.
476 L.J.Lehman, A.L.Olson, and C.J.Rebouche, Anal.Biochem., 1987, **162**, 137.

477 G.A.Qureshi, J.Chromatogr., 1987, **400**, 91.
478 J.Schmidt and C.J.McClair, J.Chromatogr., 1987, **419**, 1.
479 H.P.Fiedler and A.Playa, J.Chromatogr., 1987, **386**, 229.
480 M.Eslami, J.D.Smart, and K.A.Cohen, J.Chromatogr., 1987, **411**, 121.
481 D.E.Willis, J.Chromatogr., 1987, **408**, 217.
482 G.Blundell and W.G.Brydon, Clin.Chim.Acta, 1987, **170**, 79.
483 P.Fuerst, P.Stehle, and T.A.Graser, Infusionstherap.Klin.Ernaehr.-Forsch. Prax., 1987, **14**, 137 (Chem.Abs., 1987, **107**, 194398).
484 W.Rajendra, J.Liq.Chromatogr., 1987, **10**, 941.
485 G.A.Qureshi and P.Soedersten, J.Chromatogr., 1987, **400**, 247.
486 J.L.Tedesco and R.Schafer, J.Chromatogr., 1987, **403**, 299.
487 G.K.Svanberg, J.Periodontal Res., 1987, **22**, 133.
488 K.Kuwano, C.Sakamaki, and T.Mitamura, Nippon Nogei Kagaku Kaishi, 1987, **61**, 53 (Chem.Abs., 1987, **106**, 172203).
489 J.L.Rudy, J.C.Rutledge, and S.L.Lewis, Clin.Chem.(Winston Salem N.C.), 1987, **33**, 1152.
490 L.Montanarella, Chem.Oggi, 1987, 27.
491 H.E.Meyer, K.Swiderek, E.Hoffmann-Posorske, H.Korte, and L.M.G.Heimeyer, J.Chromatogr., 1987, **397**, 113.
492 M.M.J.O'Hare, O.Tortora, U.Gether, H.V.Nielsen, and T.W.Schwartz, J.Chromatogr., 1987, **389**, 379.
493 P.R.Young and F.Grynspan, J.Chromatogr., 1987, **421**, 130.
494 L.T.Ng, A.Pascaud, and M.Pascaud, Anal.Biochem., **167**, 47.
495 I.V.Nazimov and N.B.Levina, Chem.Pept.Proteins, 1986, **3**, 119 (Chem.Abs., 1987, **106**, 77987).
496 K.Matsumoto and S.Tsuge, Shitsuryo Bunseki, 1986, **34**, 243.
497 M.Okamoto, K.Jinno, M.Yamagami, K.Nobuhara, and K.Fukushima, J.Chromatogr., 1987, **396**, 345.
498 R.Murphy, J.B.Furness, and M.Costa, J.Chromatogr., 1987, **408**, 388.
499 H.Kotaniguchi, M.Kawakatsu, T.Toyooka, and K.Imai, J.Chromatogr., 1987, **420**, 141.
500 A.Araki and Y.Sako, J.Chromatogr., 1987, **422**, 43.
501 R.Cunico, A.G.Mayer, C.T.Wehr, and T.L.Sheehan, BioChromatogr., 1986, **1**, 8.
502 T.Naesholm, G.Sandberg, and A.Ericsson, J.Chromatogr., 1987, **396**, 225.
503 S.R.Spurlin and M.M.Cooper, Anal.Lett., 1986, **19**, 2277.
504 M.Lee, H.Nohta, K.Ohtsuto, B.Yoo, and Y.Ohkura, Chem.Pharm.Bull., 1987, **35**, 235.
505 S.Lam and G.Malikin, J.Chromatogr., 1986, **368**, 413.
506 M.Fujiwara, Y.Ishida, N.Nimura, A.Toyama, and T.Kinoshita, Anal.Biochem., 1987, **166**, 72.
507 J.Florance, A.Galdes, Z.Konteatis, Z.Kosarych, K.Langer, and C.Martucci, J.Chromatogr., 1987, **414**, 313.
508 R.H.Buck and K.Krimmen, J.Chromatogr., 1987, **387**, 255.
509 S.Einarsson, S.Folestad, and B.Josefsson, J.Liq.Chromatogr., 1987, **10**, 1509.
510 J.Wagner, C.Gaget, B.Heintzelmann, and E.Wolf, Anal.Biochem., 1987, **164**, 102.
511 J.Wagner, E.Wolf, B.Heintzelmann, and C.Gaget, J.Chromatogr., 1987, **392**, 211.
512 E.Armani, R.Marchelli, A.Dossena, G.Casnati, and F.Dallavalle, Helv.Chim.Acta, 1986, **69**, 1916.
513 S.Lam and A.Karmen, J.Liq.Chromatogr., 1986, **9**, 291.
514 G.Guebitz, J.Liq.Chromatogr., 1986, **9**, 519.
515 M.T.Aubel and L.B.Rogers, J.Chromatogr., 1987, **392**, 415.
516 D.S.Dunlop and A.Neidle, Anal.Biochem., 1987, **165**, 38.
517 K.Iwsaki, S.Yoshida, N.Nimura, T.Kinoshita, K.Takeda, and H.Ogura, J.Chromatogr., 1987, **404**, 117.
518 G.Krüger, J.Grötzinger, and H.Berndt, J.Chromatogr., 1987, **397**, 223.
519 P.Wenger and P.Javet, J.Biochem.Biophys.Methods, 1986, **13**, 275, 289.
520 M.Gleeson and R.J.Maugham, Clin.Chim.Acta, 1987, **166**, 163.
521 F.Weissbach, W.Schindler, J.Huber, and G.Kreibich, Acta Biotechnol., 1987, **7**, 377.
522 M.Hisatomi, T.Iida, T.Mitamura, K.Nagata, and K.Tomita, Denki Kagaku Oyobi Kogyo Butsuri Kagaku, 1987, **55**, 67.
523 J.L.Romette and C.L.Cooney, Anal.Lett., 1987, **20**, 1069.
524 T.Ageta. N.Mizobuchi, and H.Kodama, J.Chromatogr., 1987, **419**, 334.

525 B.Zarabian, F.Koushesh, and A.Vassef, Anal.Biochem., 1987, **166**, 113.
526 A.Csiba, Kiserl.Orvostud., 1987, **39**, 21 (Chem.Abs., 1987, **107**, 194414).
527 E.T.Backer, A.Van den Ende, and W.P.Geus, Clin.Chim.Acta, 1987, **165**, 351,
528 W.R.Frisell and D.J.Higginbotham-Willcox, Med.Sci.Res., 1987, **15**, 815.
529 S.Yamaguchi, T.Tsukamoto, and M.Senda, Bunseki Kagaku, 1987, **36**, 572.
530 J.Wu and Z.Onyang, Sepu, 1987, **5**, 236.