1
Amino Acids

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1 Introduction

The literature that is oriented towards chemistry and biochemistry of amino acids is covered in this Chapter, which has, as usual, been confined to their occurrence, chemistry and analysis. Routine literature covering the natural distribution of well-known amino acids is excluded.

Commentary on some papers is brief, so that adequate discussion can be offered for other papers where more significant synthetic and mechanistically-interesting chemistry is reported. Patent literature is almost wholly excluded but this is easily reached through Section 34 of Chemical Abstracts. The Chapter is arranged into sections as used in all previous Volumes of this Specialist Periodical Report, and major Journals and Chemical Abstracts (to Volume 112, issue 11) have been scanned to discover the material to be reviewed.

2 Textbooks and Reviews

Several books[1,2] and Conference Proceedings Volumes[3] have appeared. Reviews cover N-hydroxyamino acids,[4] distribution of D-amino acids,[5] biosynthetic pathways in plants,[6] and natural amino acids as enzyme inhibitors.[7]

Recent IUPAC-IUB Joint Committee for Biochemical Nomenclature recommendations in a number of areas including amino acids, have appeared in Journals.[8]

3 Naturally Occurring Amino Acids

3.1 Isolation of Amino Acids from Natural Sources.- This Section was introduced to this Chapter last year even though it would be thought of as a routine aspect of the literature. The generation of artefacts through extraction procedures and the ever-more-sensitive analytical methods for amino acids, clearly increase the scope for erroneous conclusions on the presence of amino acids in natural sources.

Ultrafiltration using a membrane impervious to molecules of size >2KDa allows amino acids and small peptides to be separated from proteins that have been partly degraded using poly(hydroxyethyl methacrylate)-immobilized carboxypeptidase.[9] At the smallest scale level, amino acids can be isolated from proteins that have been separated by SDS-PAGE and electroblotting on to a poly(vinylidene fluoride) membrane, excised, and hydrolysed by gas-phase hydrochloric acid.[10] At the other end of the scale, isocratic "moving-withdrawal" chromatography is advocated for separation of amino acids,[11] and isolation of amino acids as their arenesulphonate salts has been studied.[12] High recoveries of air-labile amino acids can be achieved from acid hydrolysates conducted in microcapillary tubes free from air.[13] Development of a microwave acid hydrolysis method for proteins (e.g., requiring 6-8 min irradiation of a peptide attached to a Merrifield resin suspended in propanoic acid - conc HCl, using a domestic microwave oven)[14] has been reported.

Adsorption of glycine, aspartic acid and lysine to glass beads from solutions at three different pHs has been studied.[15] Protonated lysine is adsorbed more strongly than the others from acidic solutions. A review of preparative scale ion-exchange chromatographic separation of amino acids has appeared.[16]

3.2 Occurrence of Known Amino Acids.- Significant results sifted from the continuously expanding routine literature under this heading include a distinction between racemic, therefore contemporary, coded amino acids and other amino acids more recently acquired by dinosaur egg shells,[17] and a note (in a useful review of the distribution, stereochemistry, and stable isotope composition of amino acids in fossils and modern mollusc shells),[18] of the first observation of the occurrence of DL-glutamic acid in a Pleistocene-age Merceneria fossil shell.

Non-protein amino acids in meteorites, have been argued[19] to have formed from protein amino acids after decarboxylation and deamination, rather than indicative of any particular alternative living system based on amino acids.

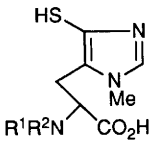
Non-protein amino acids from sources on this planet include (2S,3S)-3-hydroxyleucine, (2S,3R)-3-hydroxylysine, and Z-3-chlorodehydroalanine from HV-toxin M of the phytopathogenic fungus Helminthosporium victoriae.[20] Analogous results from higher species include the presence of β-tyrosine and N-methyl-β-bromotryptophan in Jasplakinolide, a novel antifungal anthelminthic 19-membered ketide-depsipeptide from the marine sponge Jaspis,[21] and β-D-aspartylglycine in the fish Aplysia

kurodai.[22] 4-Hydroxyisoleucine from fenugreek (Trigonella foenum-graecum) possesses (2S,3R,4S)-stereochemistry,[23] not (2S,3R,4R) as previously reported. The (2S,3S,4R)-diasteroisomer occurs in the form of its lactone as a moiety of funebrine from Quararibea funebris.[24]

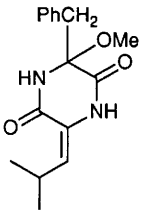
Ovothiols A-C (1) are natural π-N-methyl-4-mercaptohistidines that are shown in the pre-1986 literature erroneously as τ-methyl isomers.[25]

3.3 New Natural Amino Acids.- This heading covers derivatives and near-relatives, not only the amino acids themselves. The first example of a natural α-methoxy-α-amino acid derivative, megasporizine (2) from Penicillium megasporum NHL 2977,[26] is a member of the dioxopiperazine family, in this case a modified cyclo(phenylalanyl-leucyl). While this is not a rare type of natural product, nevertheless the phenylalanine - leucine combination is most unusual. The β-hydroxy-L-α-amino acid derivative (obafluorin, 3) is a useful broad-spectrum antibiotic.[27] A unique C_{20} β-amino acid "Adda" (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid, is a moiety of the cyclic penta- and heptapeptide cyanobacteria hepatoxins, modularin and microcystin-LR, respectively.[28,29]

3.4 New Amino Acids from Hydrolyzates.- "Dehydrobutyrine", O-methylthreonine, and N-methylasparagine are not new, but are unusual company for 14-chloro-2-hydroxy-3-amino-4-methylpalmitic acid and another nine known amino acids in pawainaphycin C, a cardioactive cyclic peptide from the blue-green alga Anabaena BQ-16.[30] Marine organisms are also represented as sources of Dolastatin 15 (a depsipeptide from the marine mollusca Indian Ocean sea hare Dolabella auricularia), that contains the hitherto unknown phenylalanine biosynthetic product (4; "dolapyrrolidone").[31] Theonellamide F, a dodecapeptide from the marine sponge Theonella contains seven common amino acids and (2S,3R)-3-hydroasparagine, (2S,4R)-2-amino-4-hydroxyadipic acid, p-bromo-L-phenylalanine, (3S,4S,5E,7E)-3-amino-4-hydroxy-6-methyl-8-(p-bromophenyl)-5,7-octadienoic acid, and a bridging amino acid, τ-L-histidinoalanine, not previously encountered in proteins.[32] A C-2 tryptophanyl - N^{im}-histidinyl linkage, with the tryptophanyl residue also linked through C-6 to the β-carbon of a substituted leucyl residue, is a notable feature of the cyclic octapeptide moroidin, from Laportea moroides, a bush prevalent in Eastern Australian rain forests.[33]



(1) Ovothiol A; $R^1 = H$, $R^2 = H$
Ovothiol B; $R^1 = H$, $R^2 = Me$
Ovothiol C; $R^1 = Me$, $R^2 = Me$



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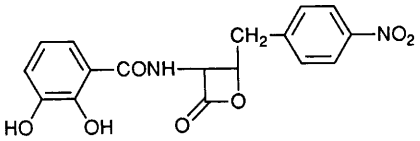

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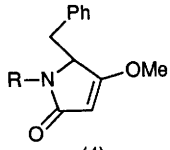

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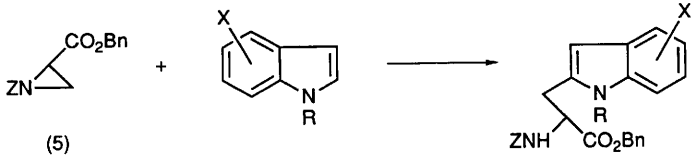

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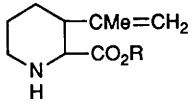

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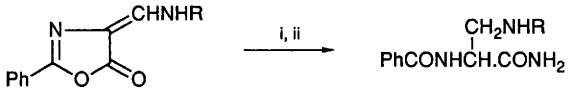
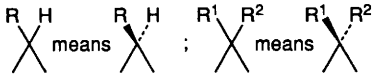


Reagents: i, $NaBH_4$; ii, NH_4OH

Scheme 1

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



4 Chemical Synthesis and Resolution

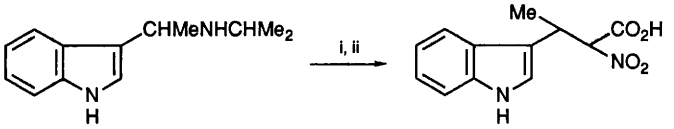
4.1 General Methods for the Synthesis of α-Amino Acids. - All standard general methods, some in new formats, are represented in the recent literature. Many of the general methods used in the area of asymmetric synthesis (next Section, 4.2) are also applicable in general synthetic routes to α-amino acids.

Reviews have appeared of aminocarbonylation,[34] synthesis of hydroxylated amino acids from epoxy- and aziridino-pyranoses,[35] and β-lactams as synthons for amino acids.[36]

Alkylation of glycine derivatives and near relatives is as popular as ever, with Schiff bases providing most of the non-routine interest. Thus, γ-allenic α-amino acids are obtained by alkylation of $Ph_2C{=}NCHLiCO_2Me$ with allenic phosphonates $(EtO)_2P(O)OCHR^1R^2CR^3{=}C{=}CR^4R^5$ in the presence of a palladium(II) catalyst.[37] Acylation of $Ph_2C{=}NCH_2CO_2Et$ with an aroyl halide after deprotonation to the delocalized aza-allyl anion $[Ph_2C{\cdots}N{\cdots}CHCO_2Et]^-Na^+$, gives N-aroylaziridinecarboxylates.[38] Chiral N-benzyloxycarbonyl aziridines have been prepared from L-serine and used for the synthesis of optically-pure benzo-substituted tryptophans (5 → 6),[39] and a similar use ("amidoethylation") of N-tosylaziridine t-butyl esters involving their ring-opening with organocuprates has been reported.[40] The route to these aziridines is through Sharpless epoxidation of allyl alcohols to give optically-active glycidic esters, these being azidolyzed and treated with PPh_3.[41] Schiff bases "the other way round" such as $Me_3SiN{=}CHR^1CO_2R^2$, prepared from the keto-acid and $LiN(SiMe_3)_2/ClSiMe_3$,[42] and $Me_2C{=}CH(CH_2)_3N{=}CHCO_2R$,[43] have been used in α-amino acid synthesis through reduction in the former case (overall, amination of a keto-acid) and trans-selective cyclization to 3-propenylpipecolic acid esters (7) in the latter case.

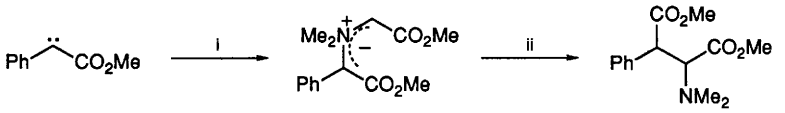
Carbonylation of amines and amides is represented by reaction of CO with carbenium immonium ions generated from N-hydroxymethylamides and imides, to give N-acylglycines, the dehydration - carbonylation process being recognisable as an extension of the Koch-Haaf reaction.[44]

N-Benzoyl-2-bromoglycine methyl ester is a well-known amino acid synthon, and undergoes substitution with alkylnitronate carbanions $R^1R^2NO_2C^-$ to give corresponding β-nitroalkylglycines, suitable substrates for elimination to "dehydro-amino acids".[45] 2-Ethoxyglycine derivatives $AcNHCH(OEt)CONHCH_2Ph$, prepared by the amidoalkylation reaction, undergo analogous substitution reactions.[46] Other standard methods are represented in the azlactone synthesis for the synthesis of β-alkylaminoalaninamides (Scheme 1)[47] and in the alkylation of methyl nitroacetate (Scheme 2) for the synthesis of β-methyltryptophan as a



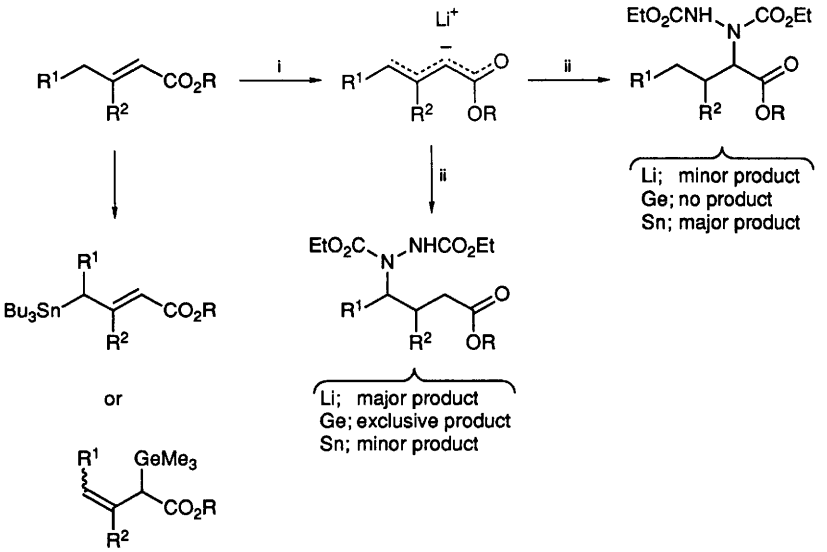
Reagents: i, $NO_2CH_2CO_2Me$; ii, H_2/Ni

Scheme 2



Reagents: i, $Me_2NCH_2CO_2Me$; ii, heat

Scheme 3



Reagents: i, LDA/HMPA; ii, $EtO_2CN{=}NCO_2Et$

Scheme 4

mixture of diastereoisomers.[48] The (2RS,3SR)-diastereoisomer crystallized out as the sole product as a result of epimerization in solution of the other diastereoisomer.[48]

Stevens rearrangement of the carbene - tertiary amine adduct in Scheme 3 is an ingenious alternative approach to using a glycine derivative in α-amino acid synthesis.[49]

Not too remote, structurally, from these glycine derivatives, is t-butyl N-(diphenylmethylene)oxamate, $Ph_2C{=}NCOCO_2Bu^t$, prepared from t-butoxalyl chloride and diphenyl ketimine. It reacts with phosphorus ylides to give "dehydro-amino acid" derivatives $Ph_2C{=}NC(=CR'R^2)CO_2Bu^t$, readily reducable to corresponding α-amino acid derivatives using sodium cyanoborohydride.[50] α-Oximino-esters $RC(=NOH)CO_2R$ are readily reduced to corresponding α-amino acid derivatives using $NaBH_4$ - titanium(III) chloride.[51]

These are novel details for standard approaches to α-amino acids, generally under the headings of amination of a carboxylic acid derivative or carboxylation of an amine. An example of the latter route is electrocarboxylation of imines with sacrificial metal anodes in membrane-free cells (e.g. $PhN{=}CHPh \rightarrow PhNHCHPhCO_2H$).[52] The "amination" approach is more widely represented, further examples including a new twist to the recently established use of azodicarboxylate esters as nitrogen source leading to very high regioselectivity in amination of lithium dienolates or Sn-masked or Ge-masked dienolates (Scheme 4) and giving α- or γ-amino acid derivatives.[53] A classical amino acid synthesis *via* α-azidoalkanoates can be completed by a one-pot conversion into the corresponding N-Boc-amino acid ester using H_2/Pd-C/Boc_2O.[54] Amination of α-keto-acids and esters is another classical route, new versions being the use of benzotriazole (BtH) for promoting the reaction of an amide with glyoxylic acid or one of its esters [$RCONH_2 + OHCCO_2Et + BtH \rightarrow RCONHCH(Bt)CO_2Et \rightarrow RCONHCH(NH_2)CONH_2$ with NH_3].[55] The transamination of phenylglycine with 2-oxoglutaric acid in the presence of N-dodecyl-pyridoxal chloride and of hexadecyltrimethylammonium chloride is the first example of mild non-enzymic transamination through the *in vivo* mechanism in the *absence* of metal ions.[56]

Less commonly-used general methods include the Ugi "Four Component Condensation" method, found to give an unexpected *cis*/*trans* distribution of products in a particular case.[57] Another route employing an isocyanide uses an aminocarbene - chromium(III) complex $(CO)_5Cr{-}CPh{=}N^+{=}CPhOCOPh$ + Bu^tNC to give C-aminoketenimines $ButN{=}C{=}CPhN{=}CPhOCOPh$, which cyclize to imidazolidin-5-ones in solution, or which add water

when treated with wet silica to give $Bu^tNHCOCPh(COPh)NHCOPh$ from which the corresponding amino acid can be obtained by hydrolysis.[58]

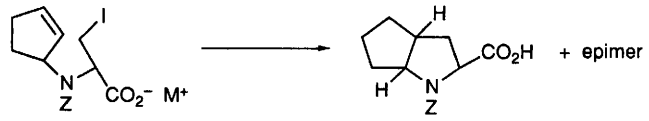
A standard hydantoin synthesis has been applied to the synthesis of 2,6-diaminopimelic acid, starting from piperidine-2,5-dicarbonitrile, and reacting it with $NH_4OH/(NH_4)_2CO_3$ at 100°C during 4 hours.[59]

At the start of this section, well-established uses of glycine derivatives in general synthetic methods for other α-amino acids have been discussed. Of course, modifications to side-chains of other simple α-amino acids should also be discussed here, insofar as they offer general synthetic routes, though a dilemma results from the way this Chapter has been organized over the years. "Specific Reactions of Amino Acids" (Section 6.3) covers such chemistry, and readers seeking coverage of this topic should scan both these parts of this Chapter in each Volume. β-Iodo-L-alanine, from L-serine, yields the corresponding alkylzinc iodide through ultrasonically-activated reaction with zinc, and then can be elaborated into 2-amino-4-oxoalkanoic acids with acyl chlorides.[60] Radical cyclization of N-substituted iodo-L-alanine derivatives using $Bu_3SnH/AIBN$ provides a route to ring-fused prolines (Scheme 5).[61]

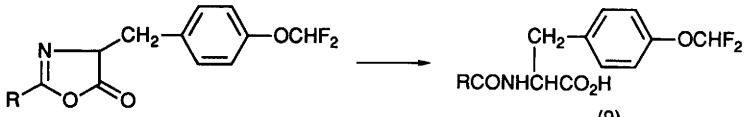
Creation of a carbanion α to the side-chain carbonyl group of β-methyl α-t-butyl N-benzyloxycarbonyl-L-aspartate[62] and the N-trityl-L-glutamic acid analogue[63] using 2.2 equivalents of lithium diethylamide (or lithium hexamethyldisilazide) at -78° can be followed by quenching with electrophiles, alkyl halides giving β-substituted aspartic acids and carbonyl compounds yielding γ-substituted glutamic acids.

4.2 Asymmetric Synthesis of α-Amino Acids.- There are some fascinating new approaches as well as equally satisfying studies that consolidate well-established methods. Several reviews[64-68] and Williams' book[2] are available. The reviews include a broad survey with 222 references,[65] some "Chemtracts" in which the work of Kunz and Pfrengle[66] and of Williams[67] is discussed, and reviews by Hegedus[68] of his own work, the use of chromium - carbene complexes in amino acid synthesis (see Vol.21, p.7).

Chirally-imprinted polymers are amazingly effective, all things considered, for some chiral recognition applications (see Section 7.5), and a crosslinked poly(styrene) imprinted through polymerization of the appropriate monomer mixture containing chiral additives, washed out from the polymer to create cavities containing salicylaldehyde and phenylboronic acid moieties, has been used in asymmetric synthesis of amino acids. The polymer-bound salicylaldehyde, converted into the salicylideneglycine Schiff base and complexed with nickel(II) ions and



Scheme 5



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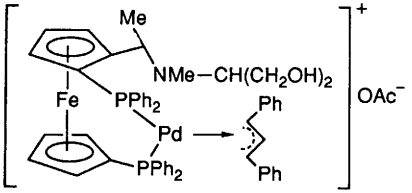

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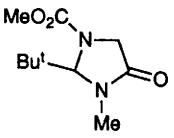

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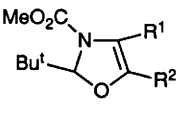

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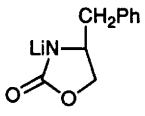

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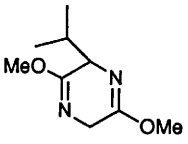

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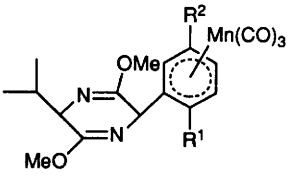

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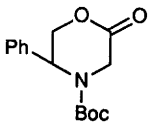

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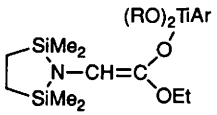


(17)

treated with acetaldehyde, gives L-threonine in slender enantiomeric excess, though 36% e.e. is obtained for a synthesis of L-DOPA on the same principle.[69]

To the familiar crop of papers reporting homogeneously-catalyzed asymmetric hydrogenation of acetamidoacrylates, using rhodium - chiral phosphine catalysts - Rh(chiral tetrasulphonated diphosphine),[70] cationic Rh(2S,4S-N-(t-butoxycarbonyl-4-[[bis(4'-methoxy-3',5'-dimethylphenyl)]phosphin]-2-([[bis](4'-methoxy-3'5'-dimethylphenyl)]methyl]pyrrolidine)$_2$ perchlorate [≡ Rh(NBD)$_2$ClO$_4$ for short],[71] for example. In the latter report, very high efficiency is claimed, but the literature on the general topic is still well-populated with disappointingly low enantioselectivities. Molecular graphics - molecular orbital calculations might come to the rescue, following an assessment through this approach, that for the system employing [Rh(S,S-chiraphos)]$^+$ X$^-$ as chiral homogeneous catalyst, 6 of the 8 possible modes of catalyzed H_2 addition to 2-acetamidocinnamates generate impossibly large atom - atom interactions.[72] A salutary warning arises in the observation that silica-bound chiral rhodium - phosphine complexes are capable of catalyzing ^1H - ^2H exchange during reaction of $^2H_2$ with (Z)-2-acetamido cinnamates in methanol.[73]

Catalytic reductive aminolysis of oxazol-5(4H)-ones also continues to disappoint in the same context, Ni - catalyzed reaction of the *p*-difluoromethoxybenzyl oxazolone (8 → 9) with H_2 in the presence of (S)-(-)-phenylethylamine giving less than 55% diastereoisomeric excess of the free acid, better than the 9 - 18% d.e. found for the same aminolysis product from hydrogenation of the corresponding azlactone and its *in situ* aminolysis by (S)-(-)-phenylethylamine.[74] On the other hand, Pd-catalyzed asymmetric allylic amination by benzylamine, of allylic substrates (RCH=CHCHRX → RCH=CHCHRNHCH$_2$Ph) in the presence of the diphosphine (10) and elaboration of the products into α-amino acids, gives better than 97% e.e.[75]

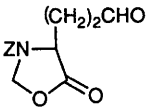
Seebach's group (*e.g.* Vol. 20, p.30) has demonstrated the high diastereoselectivity that can be achieved through alkylation of lithium enolates of 2-t-butylimidazolinones (11). Results for these substrates, derived from dipeptides by reaction with pivalaldehyde, have now been published in full.[76] A chosen enantiomer of an amino acid is obtainable through use an appropriate enantiomer of (11), and accounts of the preparation of β-arylalanines, ω-halogenoalkyl-α-amino acids, and aspartic acid, and studies of inversion of configuration by deprotonation (and α-^2H - α-^1H substitution) are given in further papers from this group.[77-79] Corresponding chiral 2-t-butyl N-methyloxycarbonyloxazolines (12; R^1 = R^2 = H, Me; R^1 = H, R^2 = CO_2Me)

can be prepared on a 100g scale from an enantiomer of serine or of threonine, a notable feature of the route being an electrochemically-operated key step.[78] These compounds undergo some standard reactions in a stereoselective mode, including Vilsmeier and Friedel-Crafts reactions at C-5 and cycloaddition to C = C (with attack from the face of the molecule remote from the t-butyl group).[79]

β-Methylphenylalanine[80] and β-methyltyrosine[81] enantiomers have been synthesised through the bromination and substitution of the chiral imide enolate (13) using the pivalic mixed anhydride of the appropriate (R)- or (S)-3-arylbutanoic acid.

The remaining papers under this heading are "asymmetric versions" of standard general methods of α-amino acid synthesis, starting either from the ingredients for constructing the -NH-CHR-CO- moiety itself, or starting from a substituted glycine. The latter group is represented by the well-established Schöllkopf synthon (14), shown to react with arene - $Mn(CO)_3$ cations to give correspondng dienyl-$Mn(CO)_3$ complexes (15) that could be regarded otherwise as D-arylglycine derivatives.[82] Another familiar chiral glycine synthon, the N-acylated 2S-phenyloxazinone (16), undergoes highly enantiospecific alkylation after conversion into its lithium enolate, as described in a most thorough overview.[83]

Aldol condensation of an aldehyde with the glycine ester - titanium carbohydrate complex (17; R' = a chiral 2-deoxyfuranose) gives D-threo-β-hydroxy-α-amino acids as predominant enantiomers. Use of the aldehyde (18) in this reaction gives (2R,3S,6S)-3-hydroxy-6-aminoheptanoic acid.[84] Synthesis of erythro-β-hydroxy-L-histidine using (R)-3-bromoacetyl-4-isopropyl-1,3-oxazolidin-2-one (19) as aldol partner for 4-formyl-τ-N-tritylimidazole has been reported.[85] Modest chiral induction is seen in condensation of chalcone with diethyl N-acetamidomalonate catalyzed by quaternary (-)-N-methylephedrinium salts to give (20).[86] Schiff bases formed with chiral carbonyl compounds are more familiar chiral glycine derivatives for use in asymmetric synthesis of α-amino acids. For example, the carbanion formed from the camphor - glycine t-butyl ester Schiff base (21) with n-butyllithium is capable of highly diastereoselective Michael addition to αβ-unsaturated esters (100% in the case of 2-alkylidenemalonates, to give anti-3-substituted D-glutamic acids).[87] Curiously, given the emphatic nature of this result, no stereoselection was observed in alkylation reactions with the norcamphor homologues.[88] In a related study of a synthesis of (S)-(+)-2,4-diaminobutanoic acid hydrochloride,[89] the chiral imine (22) provides poor levels of asymmetric induction through alkylation by $BrCH_2CN$, but 100% e.e. when Mg^{2+} or Bu_4N^+ salts are present. Synthesis



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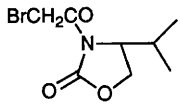

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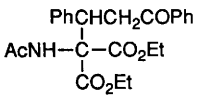

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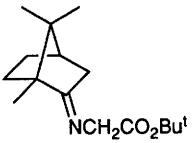

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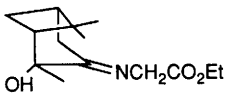

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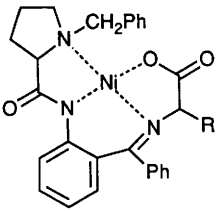

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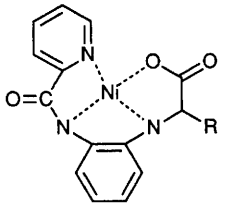

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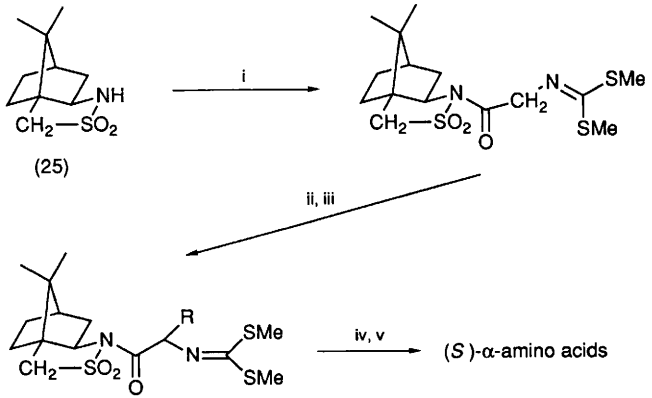


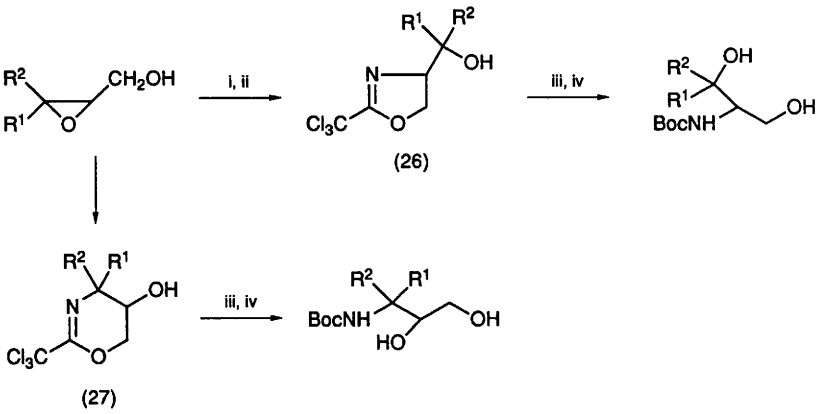
Reagents: i, $(MeS)_2C{=}NCH_2CO_2Me$, Me_3Al, toluene; ii, Bu^nLi, THF, –78°C, or $NaOH/Bu^n{}_4N^+HSO_4^-$; iii, RHal, HMPA; iv, 0.5M aq. HCl; v, aq.LiOH

Scheme 6

of α-allyl glycine has been investigated, through allylation of analogous chiral glycine imines (i.e. a chiral centre in the ester moiety as well as in the carbonyl compound used for imine formation). This involves "double asymmetric induction", by which the authors mean that the enantiomeric excess is contributed to by asymmetric induction involving two chiral centres.[90] Further development by its inventors, of asymmetric α-amino acid synthesis using the nickel-complexed glycine Schiff base (23) is described for the α-bromoglycine analogue (23; R = Br).[91] Substitution by carbanions and work-up to lead to L-aspartic acid and L-norleucine in enantiomeric purities 80 and 68%, represents some improvement over the usual technique whereby the carbanion is created in the chiral imine moiety, illustrated in the synthesis of (S)-5-(benzyloxy)tryptophan, (S)-α-allylglycine and (S)-β-(2-naphthyl)alanine.[92] Previous reports (Vol.21, p.12) have been extended further, using these synthons to give (S)-N-(α-naphthylmethyl)proline.[93] In this latter report, and in a study[94] of a new variant (24) of this synthon, alkylation of alanine analogues (e.g., 24; R = Me) has been included in an attempt to develop better routes to α-methyl analogues of common α-amino acids.

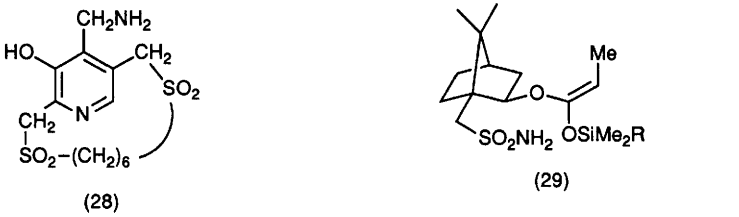
Phase-transfer alkylation of one of the simplest Schiff bases, e.g. $Ph_2C{=}NCH_2CO_2R$, in the presence of 0.2 equivalents of a cinchona alkaloid as chiral catalyst, gives 4-chloro-D-phenylalanine in >99% e.e., with 4-chlorobenzyl bromide as alkylating agent, but based on the fortuitous ease of removal of about 70% racemate by recrystallization.[95] Addition of cyanide ion to an imine derived from an enantiomer of a chiral benzylalkylamine using a cyanide - haemin copolymer complex gives α-amino acids in 80-95% e.e. after hydrolysis.[96] The e.e. is considerably higher than that of the same system in the absence of the haemin copolymer.

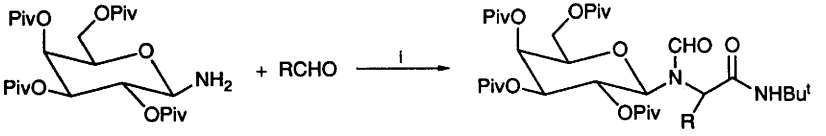
The cyclic chiral sulphonamide (25 in Scheme 6) is capable of generating 100% optically pure (S)-amino acids with return of the chiral auxiliary, when used to form the amide of the glycine-derived Schiff base $(MeS)_2C{=}NCH_2CO_2Me$, and reacted with n-butyllithium, then with an alkyl halide in HMPA.[97] Oxazolines are a masked form of Schiff base, and give optically-active β-alkyl-β-hydroxy-α-amino acids on hydrolysis when formed from α-keto-esters and methyl isocyanoacetate in the presence of a chiral (aminoalkyl)ferrocenylphosphinegold(I) catalyst.[98] Like many similar studies with initial promise, more examples are needed to establish whether there is any consistent efficiency for the process. Chiral oxazolines (26) and oxazines (27) formed from chiral 2,3-epoxyalkan-1-ols (Scheme 7) after conversion into trichloroacetimidate esters, can be converted into erythro-β-



Reagents: i, CCl_3CN/DBU-CH_2Cl_2, 0°C; ii, Lewis acid catalyst; iii, 2M-HCl in dioxan; iv, $(Boc)_2O/KHCO_3$-dioxan

Scheme 7





Reagents: i, Bu^tNC, HCO_2H, $ZnCl_2$, Et_2O-THF

Scheme 8

hydroxy-α-amino acids and α,α-disubstituted glycines, respectively, through routine oxidative cleavage.[99] An amination process is also represented in the use of the chiral pyridinophane-pyridoxamine analogue (28) for non-enzymatic transamination with 2-keto-alkanoic acids catalyzed by zinc(II) salts.[100] (R)-Amino acids are formed in modest preponderance, but a puzzling result is the finding that the macrocyclic sulphide from which (28) is prepared leads to higher e.e.s in the same process.

Photochemical amination of chiral silyl ketene acetals (29) with ethyl azidoformate gives N-ethoxycarbonyl-L-alanine esters with modest diastereoselectivity (70% as the best example so far).[101]

Use of carbohydrate templates for asymmetric synthesis of α-amino acids features in papers extending earlier studies. One report[102] describes a further use for 2,3,4-tri-O-pivaloyl-1-amino-α-D-arabinose, as the amine component of an otherwise standard Ugi synthesis (Scheme 8); another[103] involves a lengthy route from the 1,2-O-isopropylideneglucofuranose derivative (30) to β-hydroxy-α-amino acids through uneventful chemistry except for the replacement of the OH group of (30) by RNMe with the same configuration (through a double inversion sequence).

4.3 Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids.- Standard methods are illustrated for a substantial number of the current papers under this heading, and a number of papers in the preceding sections would find an appropriate place here. However, readers seeking access to this topic should, in the interests of economy with space (as in previous Volumes), expect also to have to scan the other relevant Sections of this Chapter.

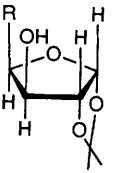
Fermentative production of protein amino acids continues to generate a substantial volume of literature, and mention is now made of representative reviews,[104] and papers on less-routine aspects. The success of a multi-enzyme system for D-amino acid synthesis with simultaneous co-enzyme regeneration is based on the high substrate specificity and thermostability of alanine racemase, and the low structural specificity but high stereoselectivity of D-amino acid transferases.[105] Access to certain amino acids through *in situ* processing of easily-available fermentation products (*e.g.* L-tryptophan formed from L-glutamic acid semi-aldehyde in 48% yield by reaction with refluxing aqueous phenylhydrazine) will become an increasingly attractive option.[106]

Natural non-protein α-amino acids include many with relatively simple structures, while others pose considerable synthetic problems.

Solutions to these problems also contribute to the development of generally applicable synthetic methods in organic chemistry, and new work meets the challenges with some style. At the simpler structural level, α-t-butyl N-Boc-L-aspartate is the starting point for a new synthesis of L-α-aminosuberic acid.[107] Only two previous (lengthy) stereospecific syntheses of this compound have been published, and these are now joined by the side-chain functional group conversion $-CO_2H \rightarrow -CON(OMe)Me \rightarrow -CHO \rightarrow -CH=CH(CH_2)_2CO_2H$ (the latter step employing the Wittig reagent $Ph_3P^+(CH_2)_3CO_2H\ Br^-$. All four configurational isomers of β-benzylglutamic acid have been prepared for screening as acyclic analogues of kainoids, starting with a serine-derived oxazoline for preparing the (2R,4R,5S)-isomer and its enantiomer, and starting with a $\Delta^{3,4}$-pyroglutamic acid derivative for preparing the other two stereoisomers.[108] Standard functional group elaboration of these starting materials is involved in these syntheses.

The apparently greater structural complexity of polyoxamic acid (2-amino-2-deoxy-L-xylonic acid) is in fact something of a benefit when the closeness in its structure to readily available monosaccharides is appreciated. Ring-opening by PhS^- of a 5-carbon hydroxylated aziridine, easily obtained from L-arabinose (Scheme 9) is a key step in new route,[109] and a nice example of a [3,3]-allylic trifluoracetimidate rearrangement is featured in another synthesis of this compound (Scheme 10).[110] Protected D-serinal (31) is a convenient starting point for such compounds, christened "glycosyl α-amino acids",[111] through elaboration of the aldehyde functional group, *e.g.* to (32) and familiar subsequent steps.

Cycloadducts formed from N-arylidene derivatives of simple α-amino acid esters with electron-deficient alkenes (*e.g.* 33 from methyl acrylate)[112] are easily elaborated into variously-substituted prolines, vying with an alternative approach, *e.g.* a synthesis of the C2-symmetric (2S,5S)-5-carboxyproline constituent of the marine alga *Schizymenia dubyi*, from (S)-1-benzyloxy-2-benzoylamino-5-hexene (Scheme 11).[113] (3R,5R)-Carbapenam-3-carboxylic acid has been synthesized from D-glutamic acid and found to be enantiomeric with the natural product from *Serratia Erwinia*.[114] Smaller and larger non-aromatic nitrogen heterocyclic α-amino acids are represented in an optimized preparation of nicotianamine by trimerization of (S)-2-azetidine-2-carboxylic acid,[115] and in a stereoselective synthesis of $\Delta^{4,5}$-pipecolic acids through a [3,3]σ-rearrangement (Scheme 12).[116] (3S)-Carboxy-(4S)-hydroxy-2,3,4,5-tetrahydropyridazine, an unusual component of Luzopeptin A, has been synthesized *via* the chiral epoxide (34 in Scheme



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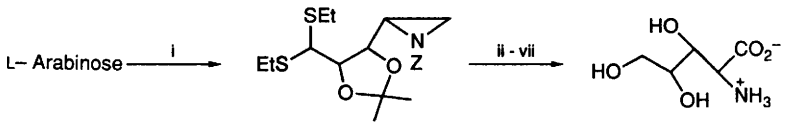
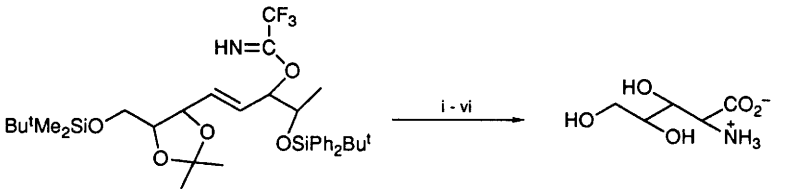
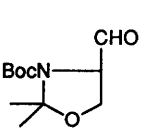


Reagents: i, protection, aziridination *via* azide; ii, PhS^-; iii, $I_2/NaHCO_3$;
iv, PhS— → $PhSO_2$—;$PhSO_2$—CH< → —CO_2H; v, $(EtS)_2C<$ →
—$CH_2OTBDPS$; vi, H_2—Pd/C; vii, aq. TFA



Reagents: i, Xylene, heat, 20 h; ii, $NaBH_4$, EtOH; iii, $(Boc)_2O$, Et_3N; iv, O_3, Me_2S/MeOH;
v, RuO_4; vi, TFA

Scheme 10



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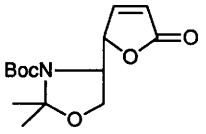

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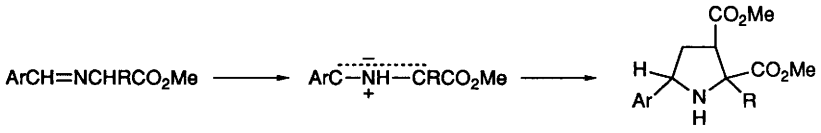

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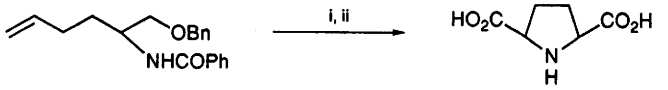
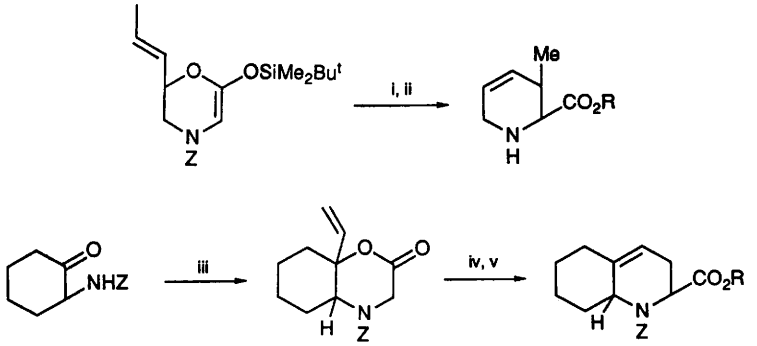


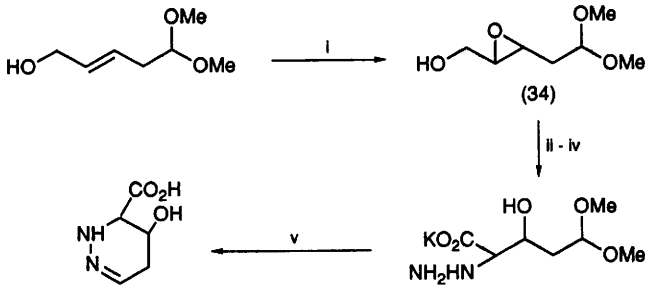
Reagents: i, I_2(3 equivalents), MeCN; ii, routine elaboration $BnOCH_2$– ⟶ HO_2C–

Scheme 11



Reagents: i, Toluene, reflux; ii, H_2/Pd-C; iii, CH_2=CHMgBr, then $BrCH_2CO_2Et$ Et_3N; then ZCl, then catalytic TsOH; iv, $Bu^tMe_2SiOCOCF_3/Et_3N$; v, toluene, reflux

Scheme 12



Reagents: i, Bu^tOOH, $Ti(OPr^i)_4$, L-(+)-diethyl tartrate; ii, RuO_4; iii, CH_2N_2; iv, NH_2NH_2/K_2CO_3; v, TFA

Scheme 13

13), a route similarly demonstrated for a synthesis of trans-3-hydroxy-L-proline.[117]

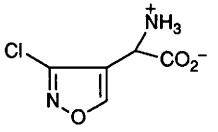
Heterocyclic side-chains are featured in some important non-protein α-amino acids, such as the isoxazole acivicin (alias AT-125) whose methyl analogue (35; Me in place of Cl) has been synthesised (as a stereoisomer mixture) from MeC≡NO and α-vinylglycine as described in a carefully scripted account.[118] A new synthesis of acivicin (AT-125) and its biosynthetic source, N^6-hydroxyornithine, can be operated so as to introduce ^2H-labelling at C-3 and C-4.[119] Diethyl aminopropyl acetamidomalonate on condensation with anisaldehyde, gives 4-MeO-C_6H_4-CH=NCH$_2$CH$_2$CH$_2$C(NHAc)(CO$_2$Me)$_2$ from which the nitrone 4-MeO-C_6H_4-CH=N$^+$(O$^-$)CH$_2$CH$_2$CH$_2$C(NHAc)(CO$_2$Me)$_2$ was prepared via the oxaziridine. Established routes lead to the named amino acids. Another acivicin synthesis study[120] applied to the preparation of analogues employs familiar steps exploiting asymmetric induction to achieve modest enantiomeric yields.

Ovothiols (1)[28] have been prepared in various standard ways, including the Schöllkopf method using (14) and the S-protected mercaptoimidazole (36) or from the corresponding aldehyde (Scheme 14).[121]

The only aromatic side-chain to be featured in this section this year appears in iso-dityrosine, a naturally-occurring crosslinking protein amino acid from cell wall glycoproteins. A straightforward synthesis from two appropriately protected L-tyrosines has been achieved through the Ullmann coupling reaction.[122] L,L-Isodityrosine has also been an attractive target for other research groups. It has been prepared through construction of the biphenyl moiety first, then to build the alanine chain on to each aryl ring (Scheme 15).[123] Use of the Schöllkopf piperazine (14) has been illustrated in a further iso-dityrosine synthesis.[124]

4.4 α-Alkyl Analogues of Protein Amino Acids.- These continue to offer useful pharmacological and enzyme inhibitory properties (e.g. α-aminoisobutyric acid offers considerable merit as a wood-rotting fungus control agent). They are accessible either through routine general methods, particularly the hydantoin route starting from a ketone, but also through Schiff bases, such as 4-Cl-C_6H_4-CH=NCHRCO$_2$Me. These undergo efficient α-arylation with (arene)halotricarbonyl chromium(II) complexes.[125] Chiral half-esters (37) of monosubstituted malonic acids yield di-anions with 2 equivalents of lithium di-isopropylamide, reactions with alkyl halides offering a versatile asymmetric synthesis.[126]

An efficient synthesis of α-trifluoromethyl-α-amino acids (see Vol.21, p.20) through Ireland-Steglich rearrangement of allyl or benzyl



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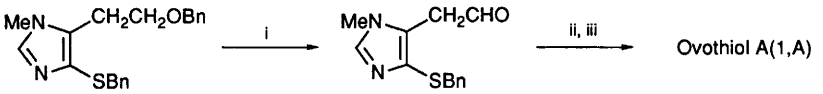
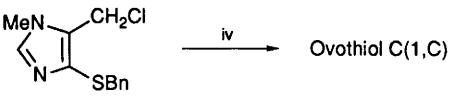
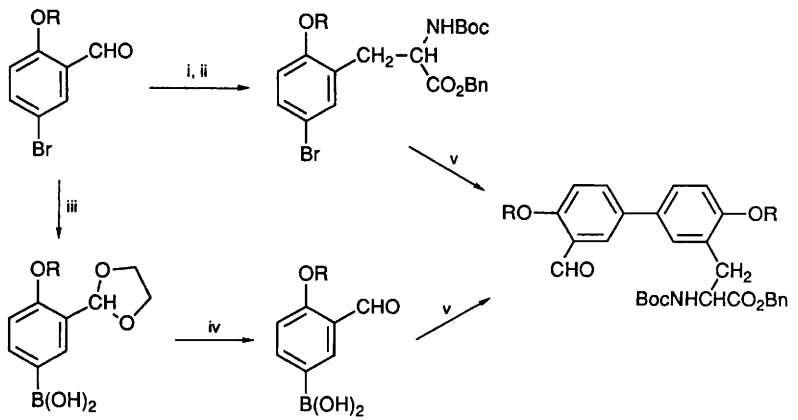




(36)

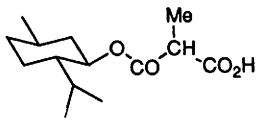
Reagents: i, BF_3/Et_2O, $HSCH_2CH_2SH$; ii, ClCOCOCl, DMSO; iii, NaCN, $Me_2\overset{+}{N}H_2Cl^-$;
iv, Schöllkopf piperazine (14), conventional elaboration

Scheme 14



Reagents: i, $BocNHCH[P(O)(OMe)_2]CO_2Bn/KOBu^t$, –60°C→20°C; ii, H_2-$[Rh(dipamp)]^+$;
iii, propane-1,3-diol, BF_3Et_2O, Mg, then $B(OMe)_3$; iv, H_3O^+;
v, $Pd(Ph_3P)_4$/THF/2M-Na_2CO_3

Scheme 15



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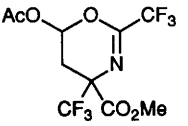

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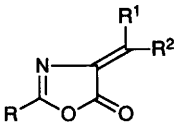

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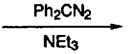

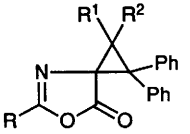

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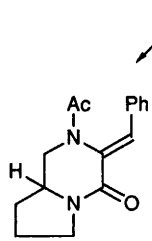

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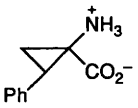
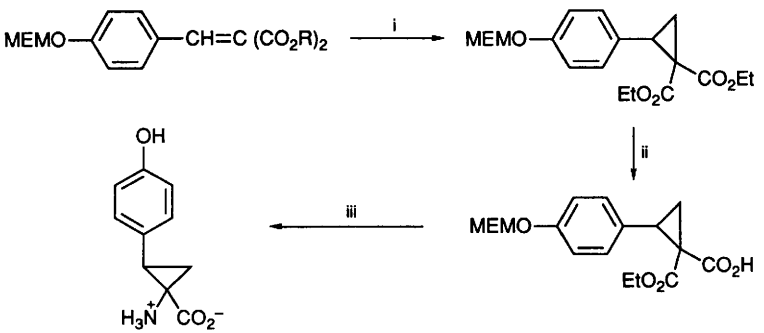


(42)

(2*S*, 3*S*, 4*R*)-isomer
(43)



Reagents; i, Me_3SOI, NaH, DMSO; ii, aq.NaOH; iii, Curtius rearrangement, deprotection

Scheme 16

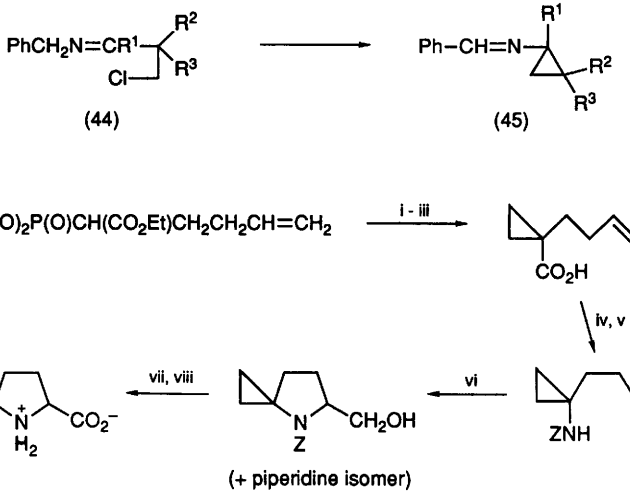
enol ethers of 4-trifluoromethyl-2-phenyloxazol-5(4H)-ones has been fully developed.[127] α-Trifluoromethyl aspartic acid α-methyl ester has been obtained from the oxazine (38), methanolysis with HCl in aq MeOH being followed by $KMnO_4$-H_2SO_4 oxidation.[128]

4.5 Alicyclic α-Amino Acids and Close Relatives.- An increasing number of examples under this heading, some known in natural sources, others found to show useful properties, is being featured in the literature.

Construction of a cyclopropyl ring on to a C = C (39 → 40),[129] (41 → 42)[130] illustrates some standard approaches. (2S,3S,4R)- and (2S,3R,4S)-α-(Carboxycyclopropyl)glycines (43 is the 2S,3S,4R-isomer) have been synthesized,[131] the latter being a potent neuroactive amino acid.[132] The routes involve the palladium(II)-catalyzed stereoselective cyclopropanation of αβ-unsaturated pyrrolidone and γ-amino-δ-lactones respectively, obtained from L-glutamic acid, as key steps, followed by routine development towards the products. The other isomers were reported on recently (see Vol.21, p.24). Another example of this growing family of "2,3-methano-amino acids" is 2,3-methanotyrosine (Scheme 16) formed as racemates of E- and Z-isomers through cyclopropanation of the cinnamate, and classical elaboration of the malonates through Curtius rearrangement.[133]

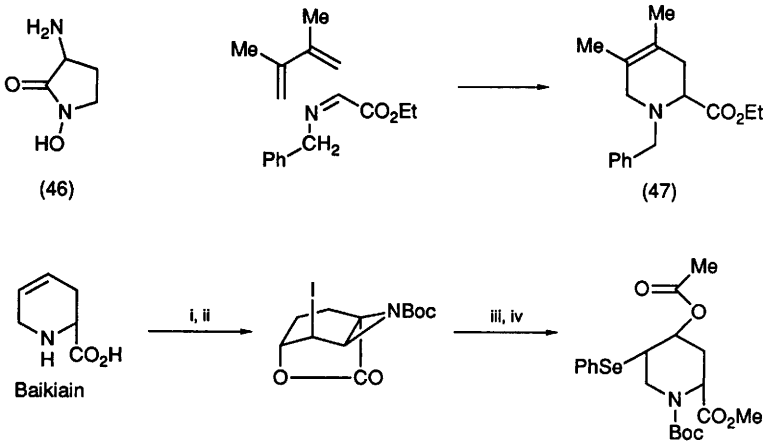
1-Aminocyclopropenecarboxylic acid has been synthesized from dimethyl diazomalonate and bis(trimethylsilyl)acetylene,[134] a previously-published method,[135] and shown to be a poor source of acetylene in plant tissue (the saturated analogue is the natural source of ethylene), but a good inhibitor of ethylene synthesis *in vivo*. Aminocyclopropanecarboxylic acid (ACC) synthase is responsible for converting S-adenosylmethionine into ACC (and is inactivated by S-adenosylmethionine during the process), while L-α-vinylglycine is a competitive inhibitor of the enzyme.[136] The C = N function (44 → 45[137]) is also amenable to cyclopropanation[137,138] (see also Refs. 38 and 109) and aziridine formation, respectively, the former case showing a reaction with chloro-imines, with the interesting feature of electron supply originating at a benzylic carbanion.[137] Aziridine synthesis has already been referred to in Section 4.1, covering "General Methods of Synthesis of α-Amino Acids".

4-Substituted prolines have been synthesized as conformationally-constrained analogues of some common amino acids, through alkylation at C-4 of α-t-butyl γ-methyl Fmoc-L-glutamate after conversion into its enolate anion.[139] A novel analogue of proline with a cyclopropyl ring grafted on at C-5 has been synthesized through the route in Scheme



Reagents: i, NaH, benzene, catalytic EtOH; ii, oxirane; iii, KOH-aq.EtOH; iv, $(PhO)_2P(O)N_3$; v, $PhCH_2OH$; vi, RCO_3H; vii, CrO_3-Me_2CO; viii, Me_3SiI

Scheme 17



Reagents: i, Boc_2O; ii, I_2/KI/H_2O/$NaHCO_3$/r.t.; iii, PhSeCl/Et_3N/CH_2Cl_2; iv, MeOH

Scheme 18

17.[140] The novel glycine antagonist 3-amino-1-hydroxypyrrolidin-2-one (46) is an exciting example of the new generation of neuroprotective agents that is emerging; it was synthesized from D- or L-methionine *via* routine elaboration of the derived S-benzylsulphonium salt.[141]

Pipecolic acid derivatives are accessible (*e.g.* 47) through aza-Diels-Alder reactions of dienes and iminium salts from benzylamine ethyl glyoxylate in DMF in the presence of 1 equiv TFA and catalytic traces of water.[142] Baikiain is a naturally-occurring, unsaturated, pipecolic acid that has been largely ignored as a chiral starting material in imino acid synthesis (and in organic synthesis more generally, perhaps). Iodolactonization (Scheme 18) followed by nucleophilic ring-opening is a good start to an active life for this compound.[143] For other examples of iodolactonization, see Ref.389.

4.6 Models for Prebiotic Synthesis of Amino Acids.- The topic has its fascination for a number of reasons, not only the need to find a credible basis for the genesis of what are now recognized as essential components for life processes, but also for unravelling reactions of amino acids subjected to different energy sources. The topic has been reviewed,[144] and familiar experiments have been repeated, often with novel variations.

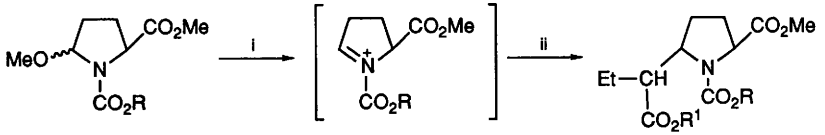
Irradiation during 5 hours, of mixtures of CO, CO_2, N_2, and H_2O by 3.0 MeV protons, simulating cosmic rays and solar flare particles, gives glycine, alanine, aspartic acid, and β-alanine, in yields higher than in all previous experiments with such mixtures (but with different energy sources).[145] Photoreduction (365 nm) of N_2 in H_2O catalyzed by TiO_2 for 1 - 4 hours at 20 - 220° to give ammonia, and the formation of glycine, alanine and serine in this mixture when acetaldehyde is added, has been demonstrated.[146] A system that is unusually sophisticated in physical terms, for this type of study, employs tunnelling spectroscopy to study events at the alumina barrier in Al - AlO_x - Pb tunnelling junctions exposed to aqueous ammonia, wet CO gas, and aqueous HCHO vapour. Spectra very similar to those of authentic glycine are obtained, very different to what is seen in the absence of a nitrogen source. For the CO - HCHO - H_2O system, spectra indicate the formation of a sugar-like polymer.[147] It has long been fashionable, and still is, to consider what precursors there may be for amino acids that are eventually formed in these experiments, and to study their behaviour under the same conditions. Thus, methyleneaminoacetonitrile $CH_2{=}NCH_2CN$ reacts with water to give glycine and simple glycine derivatives.[148]

4.7 α-Alkoxy α-Amino Acids.- Because of their value in some general α-amino acid synthetic methods, and as precursors to reactive intermediates for organic synthesis of other systems, these somewhat transient O,N-acetals (best prepared in the case of protected prolines by anodic methoxylation)[149] lend themselves to nucleophilic substitution, as illustrated in Scheme 19 for a carbapenem synthesis.[150] Phthalimido α-methoxyglycine t-butyl ester derivatives serve as starting materials in a novel Cephalosporin C synthesis (Scheme 20).[151]

4.8 Halogenoalkyl α-Amino Acids.- The simplest possible example, a protected α-fluoroglycine of known configuration (X-ray crystal structure) has been prepared by amination of a chiral fluoro-iodo-acetamide (48 in Scheme 21).[152] The value of halogen analogues of common amino acids in deceiving natural processes by causing hiatuses in metabolic pathways is illustrated by roles for αα-difluoromethyl ornithine, as an effective anti-parasitic agent (trypanosomes from tsetse fly) following apparent success established some time ago for cancer therapy.[153]

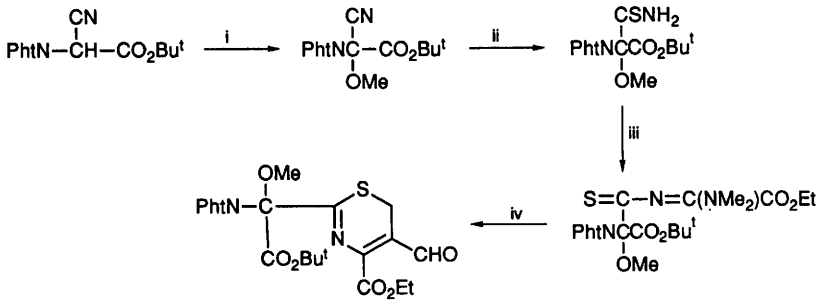
This sort of potential lies behind the synthesis of fluorinated methionines,[154] where the scope for the fluorination of the methyl group of the amino acid itself has been investigated. Methionine sulphoxide reacts with diethylaminosulphur trifluoride to give the easily-hydrolyzed monofluoromethyl analogue, and access to the di- and tri-fluoromethyl analogues is through a roundabout route: N-acetyl-DL-homocysteine + $ClCHF_2$ gives the hitherto-unknown difluoromethyl analogue, while the trifluoromethyl compound is prepared through the same route in low yield using CF_3I with photochemical activation.[154] ββ-Difluoro-γ-keto-homophenylalanine is a novel kynurenase inhibitor formed from $PhC(OSiMe_3)=CF_2$ and a protected α-chloroglycine.[155] A lengthy route is also required for the provision of (2R,3R)- and (2R,3S)-3-fluoroglutamic acids, the use of glutamate dehydrogenase assuring the delivery of these stereoisomers.[156] Claisen condensation of diethyl oxalate with ethylfluoroacetate gives $EtO_2CCF=C(ONa)CO_2Et$, α-substitution leading to $EtO_2CCH_2CF(CO_2Et)COCO_2Et$ acceptable to the enzyme for reductive amination.[156] A correction has been published[157] for the paper describing the synthesis of 3-fluoroaspartic acid.[158]

A new efficient synthesis of 3,3,3-trifluoro-alanine, permitting easy access to its α-^2H or α-^3H analogues (as do general routes, on which this synthesis is based, leading to unsaturated analogues), involves condensation of CF_3COCO_2Et with $RCONH_2$ followed by trifluoroacetic anhydride - pyridine → $CF_3C(CO_2Et)=NCOR$.[159] New efficient syntheses of 4,4,4-trifluorovaline, 5,5,5-trifluoronorvaline, 5,5,5-



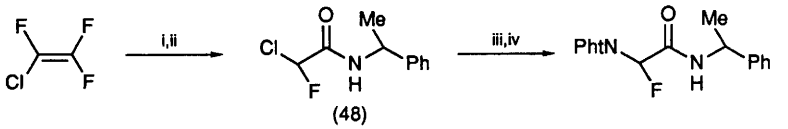
Reagents: i, Lewis acid; ii, $EtCH{=}C(OR^1)OSiMe_3$

Scheme 19



Reagents: i, NBS, $AgBF_4$, MeOH; ii, H_2S; iii, Me_2NCOCO_2Et; iv, $CH_2{=}CHO$

Scheme 20



(48)

Reagents: i; (*S*)-$PhCHMeNH_2$; ii, boiling 10% aq.H_2SO_4; iii, NaI-Me_2CO; iv, KNPht-DMF with each diastereoisomer

Scheme 21

trifluoroleucine, 6,6,6-trifluoronorleucine, 4,5,6,7-tetrafluorotryptophan, and α-(trifluoromethyl)-β-alanine use amidocarbonylation of 2-(trifluoromethyl)propanal and 3-(trifluoromethyl)propanal for the first two; and the azlactone route for the others.[160] Enzymatic resolution provides D- and L-enantiomers.

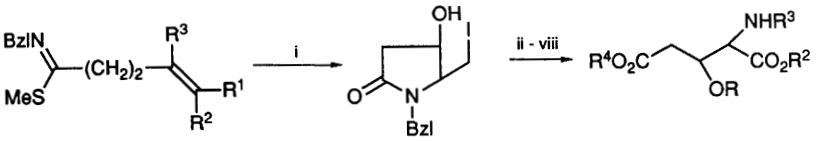
4.9 Hydroxyalkyl α-Amino Acids.- Optically-active epoxy-alkanols prepared by Sharpless kinetic resolution - epoxidation can be aminolysed and elaborated into β-hydroxy-α-amino acids.[161] An unrelated D-chirospecific synthesis of these compounds from N-benzenesulphonyl-L-serine involves addition of vinyl or allylmagnesium bromide, or (methylthio)methyl-lithium, to the carboxy group to form the corresponding ketones, then oxidation of the original serine side-chain to a carboxy group.[162] (2S,3R)-3-Hydroxyglutamic acid can be obtained through iodolactamization of γδ-unsaturated thioimidates (Scheme 22).[163]

Isomers with hydroxy groups elsewhere in the side-chain, as well as these β-hydroxyalkyl-α-amino acids, are mentioned elsewhere in this Chapter, but this section is sustained for discussion of methods capable of variation so as to constitute general syntheses. D-Xylose serves as starting material for Z-2-amino-5-hydroxypent-3-enoic acid lactone (Scheme 23).[164]

Hydroxylated 1-azabicyclo[3.1.0]hexanes feature in a synthesis of (2S,3S,4S)-3-hydroxy-4-methylproline, an echinocandin constituent (Scheme 24).[165]

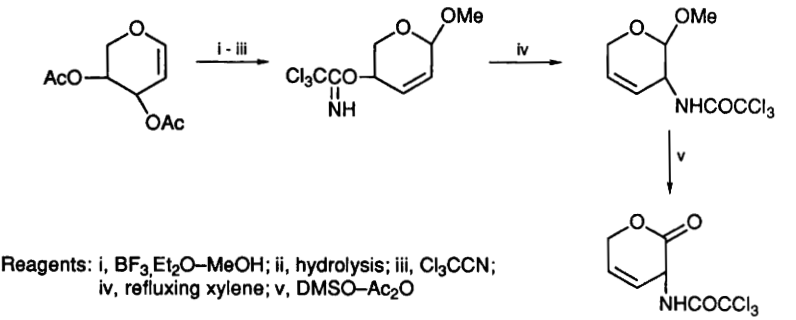
4.10 α-Amino Acids with Unsaturated Side-chains.- The chemistry of αβ-unsaturated α-amino acids ("dehydro-amino acids") has become somewhat routine, and omitted from this section (though mentioned elsewhere in this Chapter: 4.1 General Methods of Synthesis). βγ-Unsaturated analogues are more interesting in their own right, and as potential enzyme inhibitors since L-3,4-dehydro-amino acids mimic many of the conformational features of their saturated analogues. L-Vinylglycine lives up to such promise, and hearsay to the effect that it is unstable has been largely annulled. Enol ether analogues $ROCH{=}CHCH(NHX)CO_2Me$ are prepared through Wittig-type reactions of alkoxy-aldehydes with $(MeO)_2P(O)CH(NHX)CO_2Me$ followed by double bond isomerization to give the trans-isomer, using lithium di-isopropylamide followed by NH_4Cl.[166]

Other familiar functional group transformations to the same end include alkylation by aldehydes (*i.e.* aldol formation) α to the sulphonyl group in (2R)-2-Boc-amino-3-benzenesulphonyl-1-(2-tetrahydropyranyl-oxy)propane derived from L-serine (or its 2S-



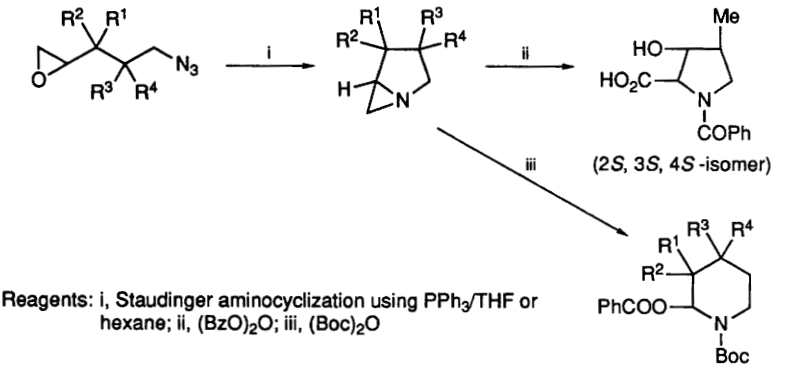
Reagents: i, I_2 –THF; ii, KOH; iii, Ac_2O; iv, TBDMSCl; v, Na/NH_3; vi, $(Boc)_2O$,NaH, CH_2N_2; vii, $Bu^n{}_4N^+F^-$; viii, O_2–Pt

Scheme 22



Reagents: i, BF_3,Et_2O–MeOH; ii, hydrolysis; iii, Cl_3CCN; iv, refluxing xylene; v, DMSO–Ac_2O

Scheme 23



Reagents: i, Staudinger aminocyclization using PPh_3/THF or hexane; ii, $(BzO)_2O$; iii, $(Boc)_2O$

Scheme 24

enantiomer), [BocNHCH(CH$_2$OTHP)CH$_2$SO$_2$Ph → BocNHCH(CH$_2$OTHP)CH(SO$_2$Ph)CH(OH)R → BocNHCH(CH$_2$OTHP)CH=CHR] followed by oxidation of the original serine side-chain to CO$_2$H.[167] Similar elaboration of N-trityl-L-glutamate esters for the same purpose[168] [using Me$_2$NCH(OMe)$_2$ as dehydrating agent, instead of Ac$_2$O as in the preceding citation]. In a paper easily accessible only through its abstract, mention is made of syntheses of L-(Z)-3,4-dehydronorvaline, L-(E)-3,4-dehydro-ornithine, and L-2,6-diamino-4-hexynoic acid.[169]

4.11 α-Amino Acids with Aromatic and Heteroaromatic Side-chains.- β-Iodo-L-alanine reacts as its organozinc derivative, with aryl iodides at 50° in the presence of bis(tri-o-tolylphosphine)palladium chloride, to give variable yields of β-arylalanines.[170] Some heteroaryl examples are included, and a parallel paper from this group[60] describes similar applications of the same derivatives.

4',α-Dimethylhistidine, a new H2 agonist, has been synthesized from 4-ethoxycarbonyl-5-methyl-3-N-benzylimidazole, through development of the ester function → -CHO → -CH=CMeNO$_2$ → -CH$_2$-CMe=NOH → -CH$_2$COMe and Bucherer-Bergs amino acid synthesis.[171]

4-Bromohomo-ibotenic acid has been synthesized in the same general routes (building the amino acid moiety on to the functionalized hydroxyisoxazoles) as used (see Vol.21, p.20) for the closer analogues to the natural product.[172]

4.12 N-Substituted α-Amino Acids.- Excluding protected derivatives for peptide synthesis, this Section is represented by N-hydroxyamino acids,[4] and by α-hydrazino acids, prepared by the base-induced conversion of diacylhydrazides ArCHBrCONHNHCOR into corresponding hydantoins, followed by work-up as for the hydantoin amino acid general synthesis.[173]

4.13 α-Amino Acids Containing Phosphorus Functional Groups.- Enantioselective synthesis of D-(-)-2-amino 5-phosphonopentanoic acid, a potential N-methyl-D-aspartic acid antagonist, illustrates further the enantiospecific alkylation of the chiral oxazinone (16), in this case by BrCH$_2$CH=CHP(O)(OEt)$_2$.[174] A recipe for the preparation of the racemate of this amino acid, using the same alkylating agent but with the Schiff base Ph$_2$C=NCH$_2$CO$_2$Et as substrate, is operated on the 50g scale.[175] The near-homologue, the "bialaphos" constituent L-phosphonotricene H$_3$N$^+$CH(CO$_2^-$)CH$_2$CH$_2$P(O)(OH)Me, has been synthesised through the Ugi route, and resolved using chymotrypsin-catalyzed

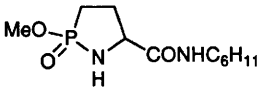
hydrolysis of its di-ethyl ester.[176] The N-hexylamide of its cyclic analogue (49) is also described in this study.

This section continues to exclude all but representative citations of amino acids in which the carboxy group is replaced by a phosphorus oxy-acid moiety. Phosphonate analogues of phenylalanine and lysine ethyl esters have been synthesized as potential serine protease inhibitors.[177]

4.14 Labelled Amino Acids.- As this Chapter unfolds, the standard methods of amino acid synthesis are encountered repeatedly in different contexts. However, these are overlaid with much ingenuity in cases where isotopic labelling must be stereospecific as well as regiospecific, for biosynthetic and other mechanistic studies.

Excellent examples are based on (2S,3S)- and (2R,3R)-[2,3-$^{2}H_{2}$]oxiranes used in a synthesis of chirally-labelled homoserine.[178] The oxiranes are prepared by asymmetric epoxidation of (E)-3-(triphenylsilyl)-2-propenol, itself prepared from 2-propynol.[179] The Schöllkopf bis-lactim ether prepared from (R)-(+)-2-methyl-3-phenylalanine and the homoserine lactone derived from $^{2}H_{2}$ and methyl (Z)-2-acetamido-4-methoxybut-2-enoate is used in a synthesis of (1S,2R)- and (1S,2S)-[2-^{2}H]-1-aminocyclopropane-1-carboxylic acids.[179] A large scale preparation of (2S,3S)-[2,3-$^{2}H_{2}$]- and (2S,3R)-[3-^{2}H]aspartic acid from labelled fumaric acids uses previously-established chemistry but benefits from the use of immobilized aspartase-containing E.coli whole cells as catalyst.[180] More routine work is covered in a preparation of DL-[4-^{3}H]glutamic acid for clavulanic acid biosynthesis studies, using the Bucherer-Bergs general α-amino acid synthesis starting from the aldehyde $MeO_{2}CCH^{3}HCH^{3}HCHO$, itself derived from the di-anion of methyl hydrogen succinate and $CF_{3}CO_{2}{}^{3}H$.[181] Preparation of DL- and L-[4,5-$^{3}H_{2}$]leucine by catalyzed $^{3}H_{2}$ addition to the corresponding unsaturated amino acids also represents a standard method for hydrogen isotope-labelling.[182] Methyl-labelled L-methionines (either $C^{3}H_{3}$- or $^{14}CH_{3}$-labelled) prepared from the appropriate iodomethanes and L-homocysteine, were used to demonstrate the presumed precursory in vivo role of methionine for S-adenosyl-L-methionine.[183]

Representative of the continuing trickle of papers describing the synthesis of ^{11}C-labelled amino acids is a one-pot preparation of [4-^{11}C]GABA using H^{11}CN (from $^{11}CO_{2}$) entrapped in KOH-THF in the presence of the aminopolyether "Kryptofix 2.2.2" for Michael addition to ethyl acrylate. A special feature, seen in the fuller accounts of this topic given in preceding Volumes of this Specialist Periodical Report, is the need to produce the final product and use it (usually for whole body tomography) within the shortest possible time, bearing in mind the



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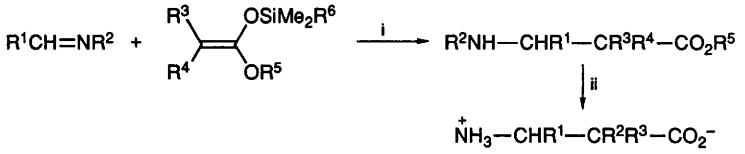
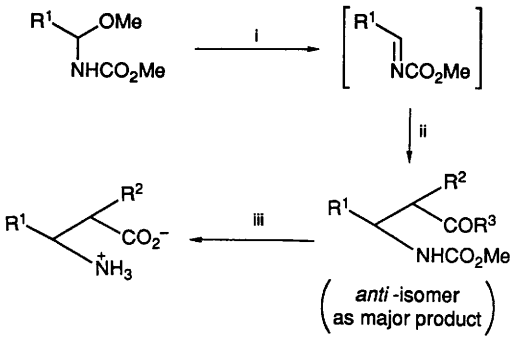


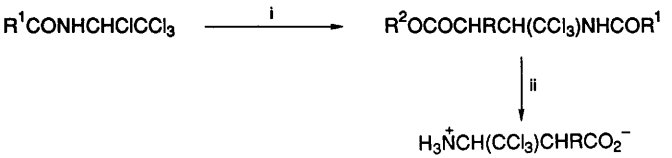
Reagents: i, $[R_3P^+]_2O(CF_3SO_3^-)_2$; ii, deprotection and H_3O^+

Scheme 25



Reagents: i, 2 equiv.LDA/THF/–78°C; ii, $R^2CH{=}CR^3O^-M^+$; iii, as ii,
with $TiCl_x(OR)_{4-x}$; (R = Pr^i, $x = 0 \rightarrow$ *syn*-isomer preferentially)

Scheme 26



Reagents: i, $RCH_2CO_2R^2$, base; ii, hydrolysis

Scheme 27

short half-life of the ^{11}C isotope. At 40 minutes for the Michael addition step in the GABA preparation, to be followed by other steps, the final radiochemical yield is low.[184] δ-Aminolaevulinic acids, $H_3N^+(CH_2)_4CO_2^-$, ^{13}C-labelled at each of the five carbon atoms, have been prepared from ^{13}C-glycine, from ^{13}C-Meldrum's acid, or from ^{13}C-sodium acetate, through standard functional group operations as appropriate for an ω-amino acid.[185]

DL-[1-^{14}C]Penicillamine is accessible from Me_2CHCHO through carboxylation of the derived 2,2,4,4-tetramethylthiazoline.[186]

^{17}O- and ^{18}O-Labelled L-tyrosines and ^{13}C-analogues of some of these, have been prepared from the O-labelled 1- and 4-^{13}C and 2- and 3-^{13}C-labelled phenols metabolized by the bacterium Erwinia herbicola.[187]

Demethylation of biosynthesized ^{35}S-L-methionine with Na-NH_3 gives ^{35}S-L-homocysteine lactone.[188]

Radiobromination of m-tyrosine gives 75% 6-bromo-isomer, the rest being an unidentified isomer.[189]

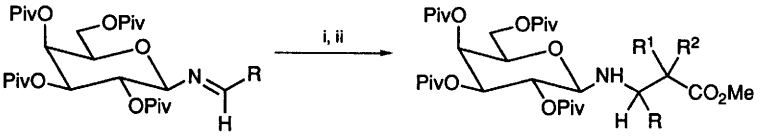
4.15 β- and Higher Amino Acids.- An increasing number of papers is being published under this heading, a trend that is especially noticeable in the year under review. The surge is not stimulated by any recent novel biological discoveries but more as a display of newly-established synthetic methodology presented as organic chemistry in its own right. Having said that, there are several biologically-important compounds under this heading, many with significant stereochemical challenges.

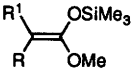
β-Amino acid esters are formed by the addition of imines to ketene silylacetals, catalyzed by diphosphonium salts $R_3P^+-O-^+PR_3$ $(CF_3SO_3^-)_2$ (Scheme 25),[190] or to other Michael receptors of a simpler type (αβ-unsaturated esters, ketones, and aldehydes).[191] N-(α-Methoxyalkyl) carbamates $MeO_2CNHCHR'OMe$ generate N-methoxycarbonylimines in situ, to add to enolate anions giving diastereoisomer mixtures (Scheme 26).[192] Benzyl vinylcarbamate undergoes concurrent alkylation (with benzyl acetoacetate sodium salt) and carboxylation (with CO) catalyzed by $PdCl_2$, to give β-substituted β-amino acid derivatives.[193] Other interesting methods of constructing these compounds have been described, α-amidoalkylation of simple alkanoates (Scheme 27),[194] and ethoxycarbonylamination with ring-opening of cyclopropane acetals (Scheme 28).[195]

Asymmetric synthesis prospects are as good for this family of amino acids as for any other, and the general imine alkylation route already mentioned[102] (Scheme 8) operates with the increasingly familiar 2,3,4-tri-O-pivalyl-1-amino-α-D-arabinose to favour the (S)-configuration at

Reagents: i, N_3CO_2Et, *h*ν, MeCN; ii, heat, DMSO

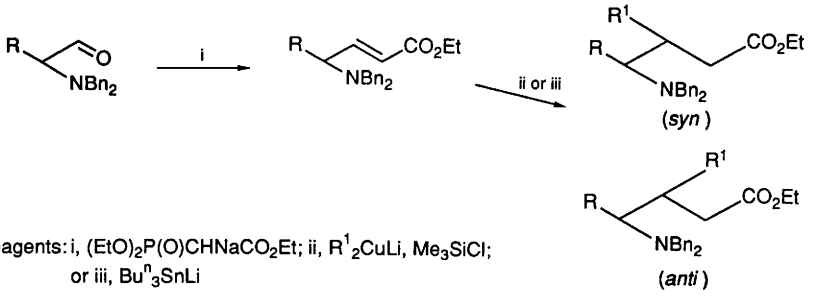
Scheme 28



Reagents: i, 

; ii, $ZnCl_2$, Et_2O, –78 °C ⟶ 30 °C

Scheme 29



Reagents: i, $(EtO)_2P(O)CHNaCO_2Et$; ii, $R^1{}_2CuLi$, Me_3SiCl;
or iii, $Bu^n{}_3SnLi$

Scheme 30

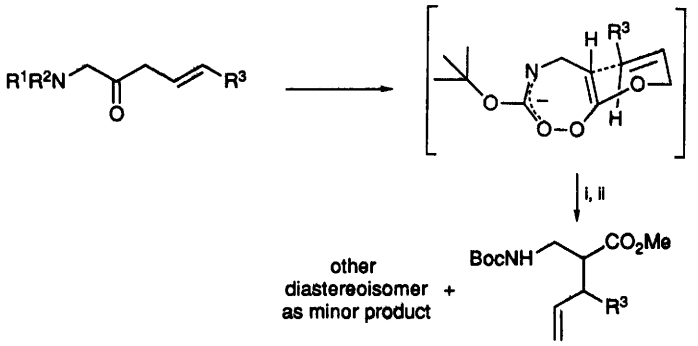
the eventual C-2 chiral centre (Scheme 29).[196] 95:5-Syn/anti-mixtures are formed by β-alkylation of alkenes formed from α-(NN-dibenzylamino)aldehydes and phosphonates $(EtO)_2P(O)CHNaCO_2Et$ (Scheme 30), amounting to a stereoselective synthesis of γ-amino acid enantiomers.[197] Alkylation was effective using n-Bu_3SnLi, but alkylation by a dialkylcuprate was unsuccessful unless Me_3SiCl was present.

Alkylation of protected β-amino acids can be accomplished to extend the available range, e.g. through lithium di-isopropylamide deprotonation followed by an alkyl halide (anti-diastereoselective),[198] or through Ireland-type Claisen rearrangement (Scheme 31) of allyl esters.[199]

Familiar natural examples that have stimulated the interest in synthesis and led to the studies described in the preceding paragraph, continue to receive the attention of those seeking sleeker synthetic routes.

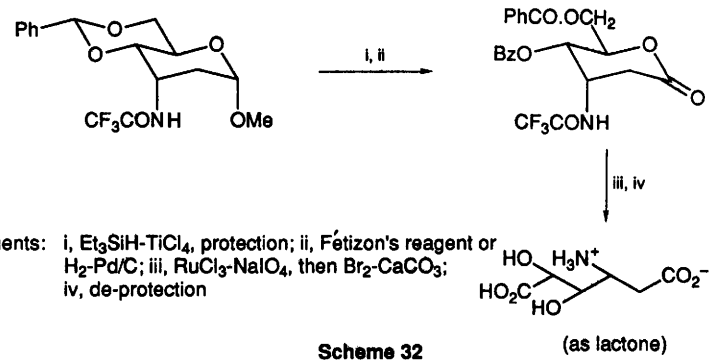
γ-Fluoro-δ-hydroxy-β-amino acids are the end result of a lengthy sequence starting with the acetaldehyde - ethyl 2-fluoropropanoate adduct, via the chain extension product (50).[200] From one point of view, this product is related to the β-amino acid moiety present in AI-77-B, the presence of three chiral centres determining the best starting point to be a protected 3-aminopyranose (Scheme 32).[201] Other naturally-occurring chiral ω-amino acids carry a hydroxylated chiral centre between the amino and carboxy groups, such as the enantiomer of 3-amino-2-hydroxy-4-phenylbutanoic acid (51) that acylates L-leucine to constitute (-)-bestatin. Two syntheses of this enantiomer are displayed in Scheme 33, one route employing the dioxalan-4-one derived from (-)-9-phenylmenthol,[202] the other starting more conventionally from Z-L-phenylalaninal,[203] and adapted so as to produce all four stereoisomers. (2R,3S)-3-Amino-4-cyclohexyl-2-hydroxybutanoic acid ("cyclohexylnorstatine") and its (2S,3S)-diastereoisomer have been synthesized, starting from N-Boc-L- or D-phenylalaninols, respectively, through established functional group transformations.[204]

γ-Amino acids are frequently of synthetic interest as analogues of GABA, the simplest member of the clan. (S)-γ-EthynylGABA and its (S)-γ-trans-butenyl analogue have been prepared by Mitsunobu inversion of $(R)\text{-}Me_3SiC{\equiv}CCH(OH)CH_2CH_2CH_2OSiPh_2Bu^t$ by phthalimide, followed by routine elaboration.[205] Amination of ω-(cycloalkanonyl)alkanoic acids through reaction with an enantiomer of phenylethylamine, followed by hydrogenation cum hydrogenolysis with H_2 and Raney nickel, gives cyclic analogues of ω-amino-alkanoic acids as potential GABA analogues.[206] Statine (52 in Scheme 34) is the other γ-amino acid featured most prominently in the recent literature, and further routes have been



Reagents: i,LDA(3 equiv.)/TMSCl/–78°C ⟶ reflux; ii, CH_2N_2

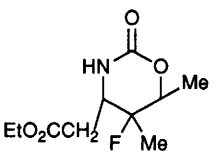
Scheme 31



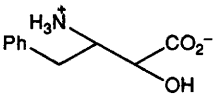
Reagents: i, Et_3SiH-$TiCl_4$, protection; ii, Fétizon's reagent or H_2-Pd/C; iii, $RuCl_3$-$NaIO_4$, then Br_2-$CaCO_3$; iv, de-protection

(as lactone)

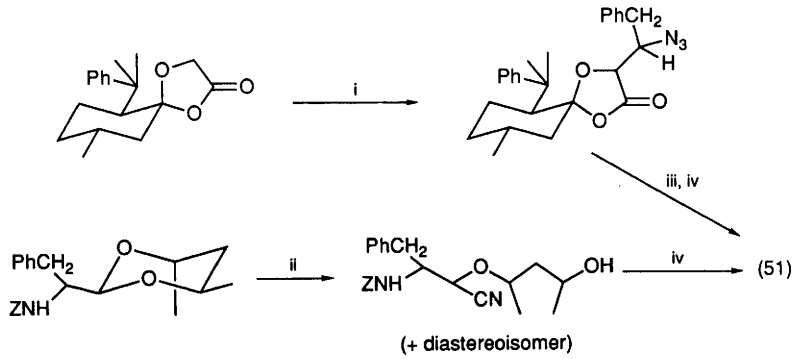
Scheme 32



(50)

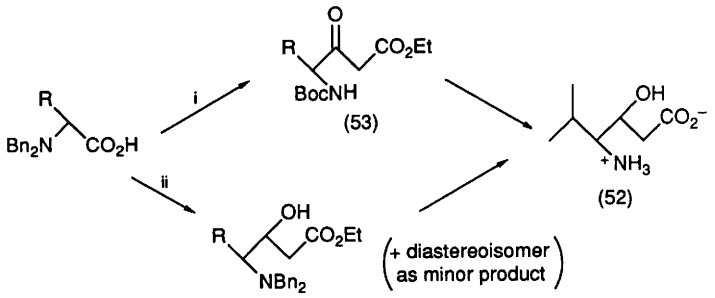


(51)



Reagents: i, $PhCH_2CHO$, base, then OH ⟶ N_3; ii, (2*S*, 4*S*)-pentanediol, $TMSCN\text{-}BF_3$, Et_2O; iii, pyridinium chlorochromate and then routine elaboration; iv, Z-removal after hydrolysis

Scheme 33



Reagents: i, *N*,*N*-carbonyldi-imidazole, then $Pr^iMgCl + HO_2CCH_2CO_2Et$; ii, $NaBH_4$-MeOH at –20°C

Scheme 34

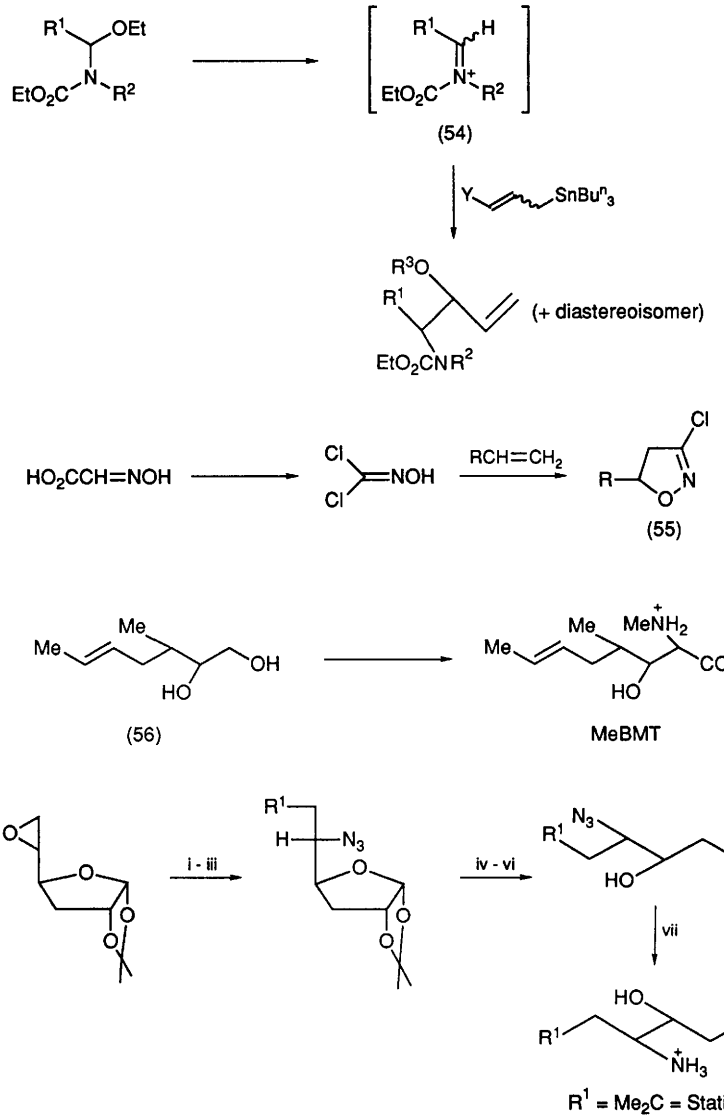
described. One of these routes starts with NN-dibenzyl-L-leucine, converted into the chiral β-keto-ester (53), which gives a 90:10 mixture of statine and its C-3 epimer on $NaBH_4$ reduction.[207] Another route is based on the addition of an allyltin reagent to the iminium ion (54) generated from the corresponding O,N-acetal.[208] Chiral auxiliaries are employed in an interesting route to (3S,4S)-statine starting from N-Boc-L-leucinal,[209] and in the "epoxy-sugar" route shown in Scheme 35.[210] An interesting one-pot general route to 4-amino-3-hydroxyalkanoic acids uses isoxazoles (55) prepared by 1,3-dipolar cycloadditions of alkenes (in the presence of $KHCO_3$) to di-chloro- or -bromo-formaldoximes (prepared from glyoxylic acid aldoxime RO_2C-CH=NOH with N-chloro- or -bromosuccinimide and Bu^tOCl).[211]

Although lengthy, the route described for the synthesis of (-)-detoxinine starting from N-Boc-D-serine is an improvement over existing routes.[212] Once again, the synthesis of the unusual cyclosporin component "MeBMT" is tackled, but to the point in Wenger's original synthesis (the 28th step!) at which the nitrogen function is to be introduced into the intermediate (56).[213]

Greater separation of amino and carboxy groups in other ω-amino acids introduces some variety in synthetic approaches, first to 5,6-diaminocaproic acid ("δ-lysine") through Bamberger imidazole cleavage of 4-(β-malonylethyl)imidazole,[214] and to (+)-galantinic acid through an Ohfune-inspired Diels-Alder addition to a protected L-serinal (Scheme 36).[215]

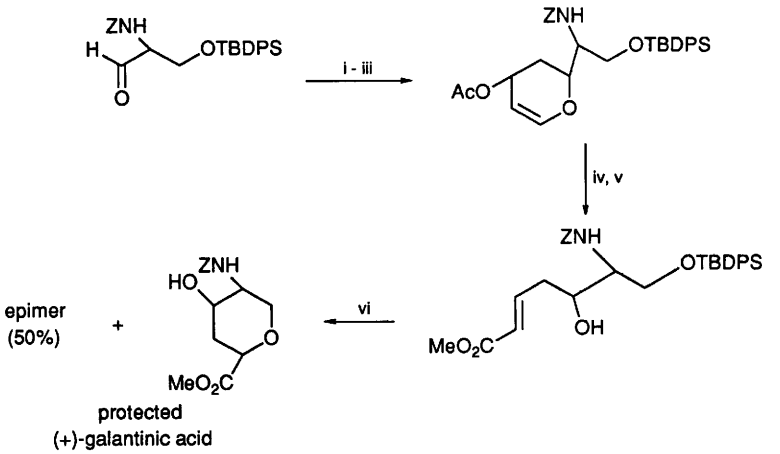
4.16 Resolution of α-Amino Acids.- This topic has an increasing number of facets and in its analytical aspects, too, covered in the later Section (7: Analytical Methods). The major division between enzymatic methods, and chemical or physical methods, determines the way that the literature is covered here.

Enzymatic methods for resolution of α-amino acids continue to be developed on traditional lines, with novel details emerging. Use of reverse micelles may have benefits for yeast-mediated resolution of N-acetylamino acid methyl esters.[216] Another "whole cell" approach describes the resolution of DL-aspartic acid using immobilized *Pseudomonas dacunhae*.[217] N-Boc-DL-α-amino acid methyl esters are substrates for thermitase-catalyzed stereoselective hydrolysis,[218] and analogous carbamate esters serve bacterial L- and D-carbamatases (with notably high selectivity: N-methoxycarbonyl-L-phenylalanine is best obtained from the former, the analogous L-alanine from the latter; NN-bis(methoxycarbonyl)-DL-lysine esters retain the N^{ε}-methoxycarbonyl group).[219] Similarly, both D-hydantoinase and D-N-carbamylase



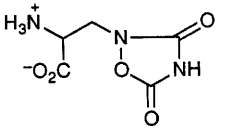
Reagents: i, R^1MgBr, CuI; ii, $MeSO_2Cl$; iii, NaN_3; iv, aq.AcOH; v, $NaIO_4$-$KMnO_4$; vi, 1 mol aq.NaOH; vii, H_2-Pd/C

Scheme 35

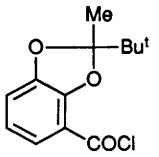


Reagents: i, $EtOCH{=}CHC(OTMS){=}CH_2/ZnBr_2/THF$; ii, $NaBH_4$, $CeCl_3$; iii, Ac_2O, Et_3N; iv, $HgSO_4$, 5M aq.H_2SO_4; v, NaCN, *etc* ; vi, K_2CO_3, MeOH

Scheme 36



(57)



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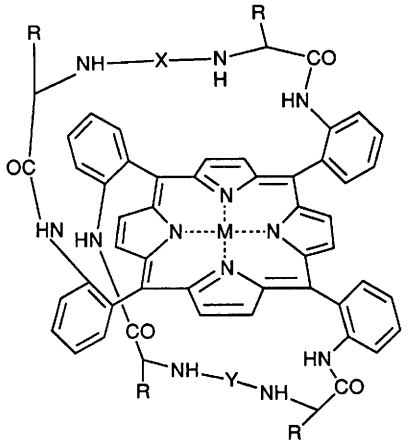


(58) X, Y = —CO—*p*-C_6H_4—CO— and other diacyl groups; M = H_2

(*Arthrobacter crystallopoietes*) are needed for the preparation of optically-pure L-amino acids from hydantoins (one of the intermediates most favoured in recent studies of large-scale resolution opportunities).[220] Racemases are useful for obtaining maximum amounts of one enantiomer from a racemic amino acid source, and mechanistic studies with pyridoxal phosphate-derived Schiff bases show that deprotonation is the initial step in the process.[221]

Immobilization of representative enzymes on activated ceramic porous alumina for these resolution approaches has been investigated.[222]

Conventional uses of enzymes have been reported for the final stages of synthesis of 2,6-di-aminopimelic acid (papain),[89] fluoro-substituted 2-amino-alkanoic acids,[160] and phosphonotricene (chymotrypsin).[176]

Application of the asymmetric transformation principle allows complete conversion of DL-cysteine into either enantiomer *via* (RS)-4-thiazolidinecarboxylic acid, using (2R,3R)- or (2S,3S)-tartaric acid and acetic acid to set up the chemical equilibrium in which this heterocycle participates. The R-heterocycle S,S-tartrate salt is least soluble, and the other diastereoisomeric salt is readily epimerized.[223] Several other examples of conventional resolution through diastereoisomeric salt formation appear in this year's literature.

Major advances can be expected in enantiomer separation through exploitation of both established and new physical discrimination mechanisms. A "three contact-point" requirement for chiral recognition is supported by molecular orbital calculations compared with experimental data for enantiomers of N-(2-naphthyl)alanine methyl ester interacting with N-(3,5-dinitrobenzoyl)leucine n-propylamide, the latter representing some currently-used species for creating chiral stationary phases for liquid chromatography (see Section 7.5).[224] Preparative-scale chromatographic resolution opportunities have been considered for β- and γ-cyclodextrins (L-dansyl-leucine complexes 62-78% more readily than its D-enantiomer with β-cyclodextrin, the variability indicating an importance of host:guest ratios).[225] β-Cyclodextrin enantioselectively catalyzes the (2,4-dinitrophenyl)ation of amino acids[226] and this offers a way of "resolving" DL-amino acids based on the separation of the N-substituted enantiomer from the reaction mixture.

Crown ethers are represented by the novel 18-6 crown, 1,2:5,6-di-O-isopropylidene-3,4-O-(1,2-bis[ethoxyethoxyl)-D-mannitol,[227] and by polymeric crown ethers containing moieties derived from L-tartaric acid.[228] (2-Aminomethyl)ated crosslinked poly(styrene) serves as stationary phase to which an L-proline moiety is bonded, which as its copper(II) complex forms another type of novel chiral polymer for

chromatographic resolution of DL-amino acids.[229] These examples tend to retain the L-enantiomer of an amino acid ester more strongly, though "tailor-made" chiral phases of these types for a given large-scale resolution must be some way into the future. This may not be so with "molecular imprints" - familiar polymers such as poly(acrylate)s prepared from the monomer containing L-phenylalanine anilide, the chiral additive being washed out from the polymer, which is then capable of efficient chiral discrimination between enantiomers of phenylalanine-containing dipeptides.[230,231] In one of these studies,[231] using perspex imprinted with L-phenylalanine anilide, enantiomers of a very close structural relative to the imprint, viz. DL-phenylalanine N-methylamide, were shown to be totally unresolved.

Other solution studies drawing on similar principles involve enantioselective transport of amino acid salts through polymeric and liquid crystal films containing chiral crown ethers[232] and ligand-exchange separation using octadecylsilica coated with NN-di-octyl-L-alanine with copper(II) ions in the mobile phase (hydrophobic amino acids retained more strongly)[233] and preparative resolution on this principle of 2-pentafluoroethyl- and trifluoromethyl-DL-alanine using D- or L-phenylalanine - copper(II) complex in the mobile phase.[234]

A small part of this section each year covers the literature on natural prebiotic enantioselection of L-amino acids, a topic that has been reviewed from the point of view of the consequences of the microscopic energy difference between D- and L-components of a racemate due to parity violation.[235] Calculations have been presented, purporting to show that L-amino acids more readily form and condense into peptides on kaolinite in its "direct" structure rather than on its less-abundant "inverse" form, thus re-presenting a familiar type of argument for the predominance of the L-series.[236]

5 Physical Studies of Amino Acids

This Section covers the major spectroscopic techniques, and other physical studies, as applied to amino acids and their derivatives.

5.1 Crystal Structures.- A neutron diffraction structure for DL-aspartic acid has been published.[237] All other studies cited here are X-ray crystal structures, i.e. lacking locations of hydrogen atoms. The structure for DL-quisqualic acid (57) reveals an unusual feature: the nitrogen atom connecting the heterocycle to the β-alanine carbon atom is held in a pyramidal configuration.[238] L-2-oxothiazolidine-4-carboxylic acid[239] and amino acid salts studied include (+)-L-arginine

di-arsenate[240] (in which L-arginine di-cations and $AsO_2(OH)_2^-$ anions form a network of hydrogen bonds), a new crystal form of L-arginine D-glutamate[241] and trimethyltin DL-glutamate.[242] Numerous amino acid derivatives (excluding peptides) have been studied: hydrochlorides of L-cysteine methyl and ethyl esters,[243] N-phosphonomethyl-L-threonine,[244] N-phosphonomethyl-L-proline,[245] N-Boc-L-tyrosine (4-bromophenacyl) ester,[246] N-Boc-alanine o-nitrophenyl ester (shown to be a rigid structure with very restricted rotation about the ester bond because one of the nitro-group oxygen atoms is wedged between the two ester oxygen atoms),[247] N-tritylglycine methylamide,[248] (-)-S-benzyl-α-hydroxymethylcysteine (as its N-benzoyl derivative; shown to possess the S-configuration in confirmation of earlier chemical correlations)[249] N-acetylamino acid amides,[250] and N-(3,5-dinitrobenzoyl)leucine n-propylamide and N-(2-naphthyl)alanine methyl ester.[251] The last-mentioned pair were studied in this way as close models of "chiral selectors" used in preparation of stationary phases for chiral h.p.l.c. (see Section 7.5); each is capable of "recognising" the chirality of the other, and it is postulated that the S-enantiomers of each of these two amino acid derivatives complex together in solution.

A review of crystal structures of amino acids, extracting data on hydrogen bonding distances and bond angles, has been published.[252]

Crystallization behaviour of amino acids has been surveyed, with particular regard to trends in, and effects of trace impurities on polymorphism (e.g. L-glutamic acid exists in α- and β-polymorphic forms).[253]

5.2 Nuclear Magnetic Resonance Spectrometry.- Specialized studies are briefly described here(as opposed to the routine, which is not). Low temperature ^{1}H-n.m.r. spectra of N^{α}-acetyl L-arginine isopropyl ester hydrochloride have been interpreted in terms of the resonances of the four NH_2 protons.[254] The puzzling dependence of the stability of S-adenosyl-L-methionine on the nature of the accompanying anion has been a long-standing problem. The molecular events in solutions at various temperatures have been concluded to involve pyramidal inversion at the sulphonium chiral centre, based on careful n.m.r. studies, and the next problem is to link this with a role for the anion.[255]

Bis-strapped chiral porphyrins (58) involving amino acid bridging units have been shown by ^{1}H-n.m.r. studies to be formed free from racemization. This illustrates in an unusual context a typical role for n.m.r. spectrometry in amino acid reactions in general, not only in the monitoring of the synthesis of small peptides.[256]

The most familiar uses of n.m.r. in the study of amino acids and their derivatives lie in the study of conformational and acid-base equilibria, and in molecular interactions in solution. The first-mentioned topic is usually well-represented here (but not this year), while the other two are represented, respectively, in the pH-dependence of ^{13}C-n.m.r. features of aspartic acid (the addition of two protons to totally-deprotonated solute is to N and O simultaneously rather than sequentially);[257] in the effect of $ClCH_2CH_2OH$ on self-association of N-acetylamino acid dimethylamides in H_2O-^2H_2O;[258] and in establishment of the site of co-ordination of methionine to lanthanide cations (La, Pr, Nd, Dy, Ho, Er, Tm) to be the carboxy group oxygen atoms.[259]

5.3 Optical Rotatory Dispersion and Circular Dichroism.- The topic, which has usually treated amino acids and their derivatives either as simple vehicles for developing general understanding of the techniques or as microcosms of proteins, has not advanced in any substantial way through the period under review. An account has been published of the use of a chiral benzoylating agent ["(+)-TBM"; 59] for derivatizing amino acids and alcohols for configurational assignments,[260] without convincing evidence for any particular advantages over existing chromophoric derivatives.

5.4 Mass Spectrometry.- Like other instrumental and spectrometric techniques discussed in this Chapter, considerable technical advances are being made, though without substantial new principles of chemistry being involved.

Negative ion (Cl^-) chemical ionization spectra of amino acids include prominent molecular ions, $[M + Cl]^-$ in the case of simple hydrophobic amino acids, and $[M - H]^-$ for polar types.[261] Positive and negative ions formed through ^{252}Cf-fission product bombardment of valine have been linked with fragmentation pathways.[262]

At a more familiar level, given the ever-widening use of the technique, fast atom bombardment mass spectra have been listed for N-benzyloxycarbonylamino acids.[263]

5.5 Other Physical Studies.- Studies that are more appropriately placed elsewhere, such as theoretical aspects of analytical techniques dependent on physical phenomena, will be found under other headings.

Topics discussed here fall under three main headings: consideration of intermolecular interactions; measurement of physical data using simple apparatus encountered in the traditional physical chemistry laboratory; more sophisticated physical measurements.

Under the first heading, discussion has been published of the relationships between the polar character and molecular dimensions of simple amino acids, and their selection for coding into proteins,[264] and a dispute has broken out over the related claim that the genetic code preferentially conserves long-range interactions between amino acids that are beneficial in some ill-defined way, between the advocate of this claim[265] and its opponents.[266] A discussion of molecular aggregation of amino acids leading to abiotic condensation and polymerization, with particular reference to the effect of chirality, is another example of armchair chemistry in this area.[267]

Micro-emulsions form with N^{α}-lauroylarginine methyl ester in aqueous media.[268] This is one of a group of papers based on interactions between amino acids and the liquid phase in which they are located; others discuss selective transport of salts of amino acid esters from aqueous media through organic membranes, with antamanide as carrier;[269] an error correction has appeared for the earlier paper from this group.[270] Hydrophobicity and surface activity data for N^{α}-dodecanoylamino acids in water have been published.[271] The effect on the structures of sodium salts of N-acylamino acids on their surface properties in aqueous solutions, has been demonstrated.[272] Interestingly, these salts show higher surface activity in solutions made with hard water, than corresponding concentrations of sodium salts of common fatty acids. A substantial study of the N^{α}-stearoyl-L-serine monolayer has appeared,[273] from the point of view of molecular recognition. Since this is a chiral monolayer, there is scope for association ("complex formation" in all its meanings) between the monolayer and one particular enantiomer from a mixture contained in the underlying solution. A similar objective is the basis of a study of the diffusion of N-(3,5-dinitrobenzoyl)-L-leucine n-butyl ester through a silicone-supported liquid membrane.[274] Oriented crystal growth of glycine has been reported at the water interface with a Langmuir-Blodgett monolayer containing L-amino acids.[275] Amino acid - receptor studies that may depend on distantly-related molecular recognition phenomena are covered in a study of the salt taste properties of amino acids, and their methyl ester hydrochlorides, in company with the dipeptide ornithyl-β-alanine.[276] Basic amino acids and their derivatives enhance the saltiness of sodium chloride but are not themselves salty. However, the human intake of Na^{+} can be cut by 75%, 50% or 25% by substituting the NaCl in a meal by the dipeptide, by glycine methyl ester hydrochloride, or by increasing the level of certain amino acids to maintain the same "saltiness", clearly a useful and presumably safe way forward for those who link sodium intake with various unwelcome consequences. At a more

scientific level, the apparent specific molar volumes of 17 amino acids have been compared with taste data to conclude that steric exclusion from taste receptors operates with certain enantiomers.[277] While on the subject of taste, side-chain mono-esters of aspartic and glutamic acids are repellent to oriental weatherfish (Misyornus anguillicaudatus), but the di-esters are not, indicating a leading taste role for the terminal carboxy group.[278] The marked anti-amnaesic effect of D-pipecolic acid in rats has been reported, a property for which the peptide H-Pro-Leu-Gly-NH_2 is already known.[279] Apparent partial volumes of 11 amino acids in water at 15-55°C[280] and similar properties related to infinitely dilute aqueous amino acid solutions[281] including effects of various salts on structure-making and structure-breaking in aqueous glycine solutions,[282] and adiabatic compressibility of aqueous methanolic solutions of amino acids[283] have all been determined by densitometry.

Amino acid first and second thermodynamic dissociation constants have been given precise values for 0.1 mole fraction 1,2-propanediol - water solutions of glycine, through e.m.f. measurements based on the silver chloride - hydrogen electrodes.[284] Calorimetric measurements of the same data have been extended to third dissociation constants of acidic amino acids (glutamic and aspartic acids, tyrosine),[285] and also encompass other hydroxyl- and fluorine-substituted phenylalanines.[286] This extensive study includes discussion of the effects of amino acid side-chains on the magnitude of dissociation constants.[287] Dissociation constants for DL-alanine, DL-valine, L-valine, and DL-leucine in aqueous dioxan have been determined.[288]

Calorimetry studies of N-acetylamino acid amides yield excess enthalpy data.[289] Volume changes accompanying ultrasound absorption by L-cysteine in aqueous solutions have been interpreted for the first time in terms of proton transfer reactions and individual ionization rate constants.[290] Differential thermal analysis data lead to estimates of kinetic, stability of amino acids (Met < Ser ≃ Arg < Arg,HCl) and corresponding methyl ester hydrochlorides (roughly the same order).[291] The thermal stability of asparagine from these data is greatest in aqueous solutions at pH 5 - 7, but its stability diminishes in the solid state as the proportion of water of crystallization increases.[292] A thermodynamic study of the interaction energy between alkylureas and L-valine, L-valyl-L-valine, L-leucine and L-leucyl-L-leucine respectively, has been reported.[293]

More sophisticated instrumentation provides Raman spectrometric data for tryptophan and valine (D, L-, and DL-),[294] and a series of substituted tryptophans.[295] The newer generation of Raman studies employing polarized radiation has included studies of aliphatic amino

acids in water and in ^2H_2O at pH values appropriate for predominance of zwitterionic and deprotonated forms, respectively.[295] Features of intermolecular vibrational coupling in solid glycine have been identified.[297]

E.s.r. Spectrometry of irradiated amino acids is featured regularly in the literature, this year represented by variations in $^\alpha$H-hyperfine splitting as a function of temperature for X-irradiated solid L-alanine.[298]

Undoubtedly, the star paper this year has to be the account of the visualization of individual amino acid molecules adsorbed on highly orientated pyrolytic graphite, using scanning tunnelling microscopy. The amino acids are "seen" (probably through a charge transfer mechanism) to cluster, in pairs for leucine, methionine, or tryptophan, but in larger clusters for glycine.[299]

5.6 Molecular Orbital Calculations.- These methods have a role to play in conformational studies in particular, and have been applied in this context to N-acetyl-L-serine methylamide,[300] serine phosphate,[301] and the cyclosporin constituent "MeBMT", the threonine derivative (2S,3R,4R,6E)-3-hydroxy-4-methyl-2-methylamino-6-octenoic acid depicted beside formula (56).[302] The particular context for the last-mentioned study is the elucidation of the effects of change of chirality and of further substitution on the side-chain conformation of this important amino acid.

6 Chemical Studies of Amino Acids

The organisation of this section follows the pattern of preceding Volumes, with an account of racemization separated out as the starting section, due to the importance claimed for it, for assignments of age to fossils.

6.1 Racemization.- There is more of a spread in this, than in any other Section of this Chapter, from thoughtful science to somewhat whimsical applications based on suspect principles. At the former extreme, rates of racemization at pH 8, 140°C, of 13 α-amino acids with functional groups in their side-chains have been compared, with some simple amino acids with alkyl side-chains included as standards. The hydroxyalkyl amino acids showed most racemization, with a decreasing order methionine, alanine, aspartic acid and glycine, aminoadipic acid, and pyroglutamic acid for certain others.[303] Racemization of amino acid residues during alkaline hydrolysis of casein, oat protein, or rapeseed

protein gives a somewhat similar order of decreasing level of racemization: serine, aspartic acid, phenylalanine, glutamic acid, and valine.[304] However, such data do not separate the propensity for racemization of a residue when part of a protein, from that of the free amino acid, a factor that is important as shown in spectacular fashion by the generation of nearly 70% of the D-enantiomer of proline from the tetrapeptide L-prolyl-L-leucylglycylglycine heated at 148.5°C at pH 6.8 during 90 min. This must mean that some cleavage into dioxopiperazines was occurring and that the neighbouring amino acid(s) residue affect(s) the racemization rate at a particular chiral centre.[305] A similar result was found for the racemization of aspartic acid and asparagine residues in human myelin basic protein, where highest rates were seen for these residues when adjoining arginine.[306]

These reasons for being suspicious of age determinations based on amino acids enantiomer ratios have been aired in preceding Volumes of this Specialist Periodical Report. Another obvious problem is lack of knowledge of the catalytic effect on racemization rates, of any or all the compounds in the immediate vicinity of the racemizing centre. But none of this has dissuaded continuing applications. Teeth dentin from sound teeth can be dated to within four years either way from the known age, based on the D:L-aspartic acid ratio (but not for "abnormal" specimens);[307] darkened teeth from "burned bodies" show a more advanced age.[308] Good scientific practice is shown in a study in which bones from three 12th Century German burials were used to calibrate results for the corpse of Emperor Lothar I, to determine that it was boiled in water for about 6 hours before burial. Evidently, boiling was decided upon to avoid post-mortem decay since the body was to be buried at the Imperial castle 500 Km away from the place of death.[309]

A classical technique for amino acid racemization employs Schiff base formation and is based on the enhanced prototropy in such derivatives. This is particularly rapid using an ordinary domestic microwave oven (≈ 2 min) in a Teflon vial reactor (50 mg amino acid, 1 cm^3 AcOH, 50 μL PhCHO under N_2; the method is even more effective using trifluoroacetic acid).[310]

6.2 General Reactions of Amino Acids. - This section covers reactions that apply to amino and carboxy groups of amino acids, either reacting separately or simultaneously, and therefore are general in character (even though they may be exemplified only for representative amino acids).

Pyrolysis of 18 amino acids in the inlet port of a gas chromatograph, has been carefully standardized for the purposes of pyrolysis-g.l.c.

analysis, but the results are of general relevance in many contexts; from the proper handling of analytical samples to food chemistry, among others. The volatile products identified for protein amino acids are: alanine (acetaldehyde), leucine (3-methylbutyronitrile and 3-methylbutanal), isoleucine (2-methylbutyronitrile and 3-methylbutanal); valine (2-methylpropionitrile); phenylalanine (toluene); proline and hydroxyproline (pyrrole); tyrosine (phenol and p-cresol); tryptophan (indole and skatole); glutamic acid, glutamine, aspartic acid, asparagine (unknown).[311] There is much variation in results from experiments such as these,[312] as shown by a study of proline pyrolysis, forming "the aroma substance" 2-acetyl-1-pyrroline under "bread-making conditions".[313] An area of chemistry that is opening up for amino acids, as for other classes of compound, is the consequences of sonolysis. Radicals are generated in concentrated aqueous solutions due to peroxide formation, and additional radicals are formed from amino acids due to high temperatures created around collapsing cavitation bubbles. The resulting chemical changes have as yet received little attention.[314]

Routine studies of oxidative degradation of amino acids by familiar oxidants (Chloramine-T,[315] acid $KMnO_4$,[316] cerium(IV) sulphate,[317] and potassium periodate[318]) can be indicated by a few representative citations, but more interesting results with an analytical context are described for electrochemical oxidation of N-toluene-p-sulphonylglycine and dansylglycine.[319] During Fenton oxidation (Fe^{2+} - H_2O_2) of amino acids, leucine gives 3-methylbutanal (isovaleraldehyde) and α-ketocaproic acid; CO_2 evolution is stimulated by Fe^{2+} and ADP and unusual Fe - amino acid complexes seem to be involved. Oxidation is maximal when the iron-chelated amino acid is in the presence of free Fe^{2+} ions or a second type of iron complex.[320]

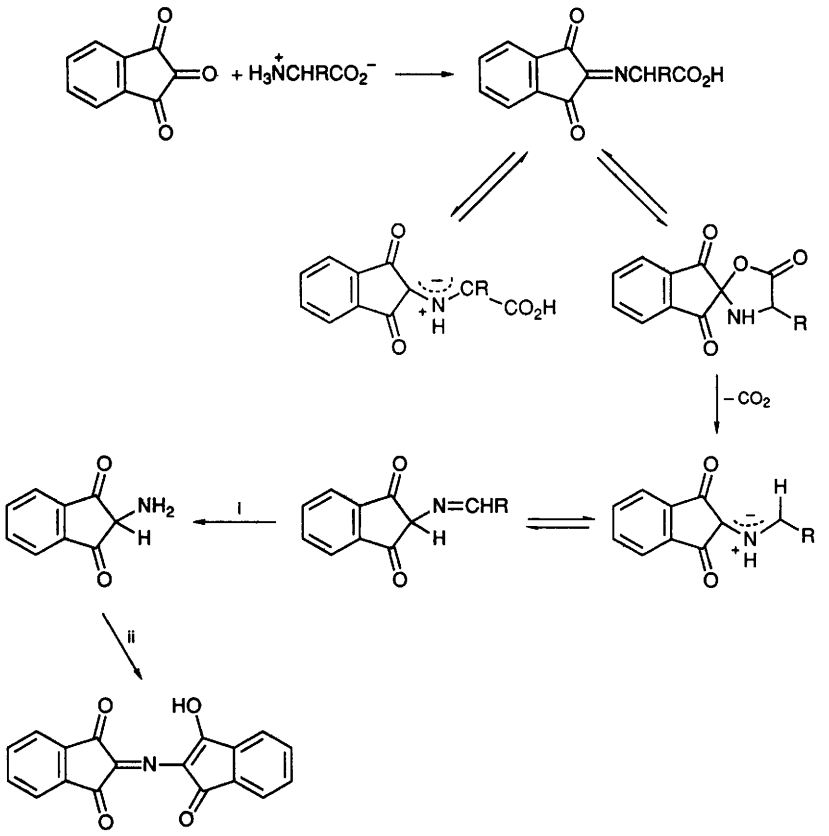
Amino acids react with carbonyl compounds to give Schiff bases, but what is known as the Maillard reaction is the consequence of a number of subsequent reactions made more complex by the fact that the carbonyl compound is a monosaccharide. A deceptively simple model, the reaction between glycine and glyoxal (a 2:1-molar mixture heated in boiling water during 24 hours), leads to products formed by the involvement of formaldehyde (the Strecker degradation product of glycine) and of methanol (the product of the Cannizzaro reaction with formaldehyde). Thus, 2,4,6-trioxaheptane and 2,4,6,8-tetraoxanonane are formed.[321] This reaction is evidently not a suitable model for the Maillard reaction, through which pyrrolecarbaldehydes, pyridines and pyridazines are the most significant products. Four pyrazines have been detected in the reaction mixture formed between glycine ethyl ester and

glyceraldehyde,[322] and 2,3,5-trimethylpyrazine is the most abundant product from glycine, and 2-methylpyrazine from arginine, when these amino acids are reacted with glucose.[323]

Reviews of the Maillard reaction[324] include its course under physiological conditions.[325]

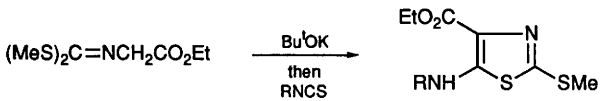
Imine formation, as briefly referred to in preceding paragraphs (racemization; Maillard reactions) is continuing to develop into other profitable mechanistic and synthetic areas of study, mainly due to their prototropic rearrangement into azomethine ylides. A lengthy review has been pubished on the author's work on the formation of these ylides and their cycloaddition reactions,[326] but substantial contributions continue to be made also by Grigg's group. A convenient alternative route to these Schiff bases is to react a primary amine with an α-keto-acid, and Strecker decarboxylation (in boiling benzene for example)[327] gives the azomethine ylide that can be trapped by sulphur (to give a secondary thioamide), or by alkenes through cycloaddition.[328] One area of interest generated by the intermediacy of these dipoles is in properties of pyridoxal imines in relation to metabolic transamination processes. Also, dipolar intermediates have been implicated in the Strecker degradation, as represented by ninhydrin oxidation (Scheme 37), and the textbook mechanism has needed revision,[329] since azomethine ylides of two different types can be trapped by cycloaddition to maleimide. Imines formed in hot DMF between α-amino acids and pyruvic acid, ethyl pyruvate, or pyruvaldehyde undergo decarboxylation, ylide formation and stereospecific cycloaddition to an alkene present in the reaction mixture.[330] It is suggested that pyruvate-dependent carboxylases may react <u>in vivo</u> through azomethine ylide intermediates. Four new classes of tandem Michael addition and 1,3-dipolar cycloaddition have been identified for imines derived from glycine ethyl ester and aminoacetonitrile in the presence of lithium salts (favouring the Michael addition route) and triethylamine.[331] Similar results have been published for N-benzylidenealanine methyl ester.[332] Acyliminium salts formed from N-alkylpyroglutamic acids through decarboxylation using P_2O_5 and methanesulphonic acid can be arylated to give 5-aryl-N-alkylpyrrolidin-2-ones.[333]

Other uses for amino acid Schiff bases also illustrate consequences of α-carbanion formation (on which their use in a general method for α-amino acid synthesis depends; see Section 3.1); in an example (60) given here, carbanion formation is exploited in synthesis of five-membered heterocycles.[334] Support for the oxazolidin-5-one intermediate proposed by Grigg (Scheme 37) is mentioned in the account of



Reagents: i, H_3O^+; ii, indan-1,2,3-trione

Scheme 37



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pyrrolidine synthesis from formaldehyde, an amino acid, and an electron-deficient alkene (dimethyl fumarate give trans-3,4-dimethoxycarbonylpyrrolidine).[335] The azamethine ylide is involved in a synthesis of optically-pure cis- and trans-2,5-disubstituted pyrrolidines from N-benzylidene-amino acid esters by cyclisation of the corresponding alkanols formed by borohydride reduction,[336] and is an intermediate in the cycloaddition of N-arylidene-amino acid esters to nitrosobenzene to give a diaryl nitrone and an α-imino-N-(1-alkoxycarbonylarylidene)arylacetamide.[337] Pyrroloquinolinequinone (PQQ) reacts with amino acids with uptake of oxygen, to yield a yellow compound, considered to be an oxazole formed from a Schiff base via an oxazoline,[338] and explaining the inactivation of "quinoproteins" (PQQ-enzymes) by ammonium salts.

Simple reactions at the amino group include its substitution as a complete unit by chloride by diazotisation. This occurs with retention of configuration with 95-98% e.e. for alanine, valine, leucine, isoleucine, but less hindered side-chains lead to more racemization).[339] Reactions in side-chains that accompany diazotization have been investigated, ornithine, citrulline and arginine giving 5-membered heterocyclic products, cysteine giving thiiranecarboxylic acid and lactic acid sulphate, and cystine undergoing disulphide cleavage.[340] Bromine water and glycine are presumed to yield an equilibrium mixture of N-bromo- and NN-dibromo-glycine, these reacting with hydrogen peroxide so as to generate O_2 and, again presumably, to return the amino acid. These inferences were drawn so as to support a proposed reaction of peroxidase with $Br^- \rightarrow Br_2$ and a roundabout route to the production of oxygen through intervention of amino acids.[341] Selective N-phenylation of α-amino acid esters with triphenylbismuth di-acetate catalyzed by Cu^o or a copper(II) alkanoate gives mono-N-phenylamino acid derivatives in all cases except glycine ethyl ester, which gives a mixture of N-monophenyl- and NN-diphenyl derivatives.[342] Histidine and arginine derivatives do not react.

A simple improved preparation of N-tritylamino acids is somewhat wasteful of trityl bromide, since N-tritylamino acid trityl esters are formed first and cleaved in situ with MeOH at 50°C.[343] Other N-protecting groups studied include 2-[4-(methylsulphonyl)phenylsulphonyl]-methoxycarbonylamino acids.[344] These are alkali-labile on the β-elimination principle, but resist catalytic hydrogenolysis and are somewhat more stable than Fmoc-analogues. Enamines formed by reaction of amino acids with 9-(hydroxymethylene)fluorene (in equilibrium with 9-formylfluorene) are claimed on the basis of representative examples to be more protective towards racemization than well-known urethane

protecting groups.[345] Routine but useful preparative work covers the preparation of pure Fmoc-amino acids and other urethanes using O,N-bis-trimethylsilyl amino acids.[346] "Pure" in this context means free from Fmoc-oligopeptides, formed in a well-known side-reaction that accompanies Schotten-Baumann acylation at the amino group when the carboxy group is unprotected.

The formation of substituted iso-indoles from the reaction of amino acids with o-phthaldialdehyde and a thiol has been confirmed by n.m.r. studies with glutamic acid, glutamine, and aspartic acid, for the case where the thiol is glutathione.[347] Though there may appear to be no special reason why a complicated thiol should be used when a simple one will do, isoindoles formed using N-acetyl-L-cysteine are much more stable than corresponding compounds prepared using mercaptoethanol.[348]

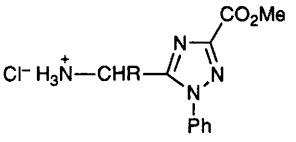
As already indicated, some reactions at the amino group are followed by further processes involving other sites in the molecule. N-Phenylthiocarbamoylamino acids are believed to require fairly drastic treatment in order to undergo cyclization and rearrangement to N-phenylthiohydantoins (PTHs; the basis of the booming analytical process involving these "PTC-amino acids"), in spite of the inability to isolate PTC-amino acids on the part of Edman himself[349] and others (but see Section 7.5), because these compounds readily cyclized to PTHs. It is now found that sulphur-containing cyclic amino acids form PTHs spontaneously through reaction with phenyl isothiocyanate.[350] Reference to the corresponding reaction with pipecolic acids[351] shows that the course of this reaction is not so simple with cyclic imino acids as might be thought.

The PTC-amino acid, in fact, cyclizes in acid media to form a 2-anilinothiazol-5(4H)-one, and this is usually required to rearrange to the PTH in the normal operation of the Edman sequence analysis of peptides. However, ring-opening to form a PTC-amino acid ester through use of an alkanol avoids the stringent need to remove traces of acids for clean PTH formation, and gives an easily analyzed derivative.[352] N-(N-Benzyloxycarbonylaminosulphonyl)amino acids $ZNHSO_2NHCHRCO_2H$ formed through reaction of chlorosulphonyl isocyanate, benzyl alcohol and an amino acid hydrochloride, cyclize to 1,1-di-oxo-1,2,5-thiadiazolidin-3-ones after Z-cleavage, cyclic sulphonamides that were found after tasting (through accident or misunderstood instruction?) to be not sweet.[353] Boroxazolidinones, cyclic mixed anhydrides formed between 1,1-diphenylborinic acid and an amino acid or an N-monoalkylamino acid, are specifically suggested to offer a means of analyzing amino acids in admixture with peptides and proteins.[354] 2-Oxazolidinones are formed from amino acids and $Cl_3COCOCl$ ("diphosgene") after BH_3-SMe_2-BF_3

reduction of the carboxy group to give 2-amino alkanols without racemization,[355] and the carboxy group is also involved in a preparation of chiral triazoles (61) by reaction of Z-amino acid mixed anhydrides with methyl phenylhydrazono esters, followed by Z-removal.[356] More continuing interest is likely to be found in the formation of the Meisenheimer adduct (62) from N-methyl-N-(2,4,6-trinitrophenyl)glycinate anion, the first example of participation by the carboxylate anion in such systems,[357] and for the formation of lactams from β- and higher ω-amino acids using triethylgallium in benzene.[358] A cycloaddition route to 3-(N,N-disubstituted)amino-β-lactams uses imines RN=CHR' with zinc enolates of N,N-disubstituted amino acid esters formed by deprotonation with lithium di-isopropylamide and treatment with $ZnCl_2$.[359] Optically-active oxazol-5(4H)-ones are formed from N-acylamino acids and EEDQ or IIDQ;[360] and from L-tryptophan the optically active 2-trifluoromethyloxazol-5(4H)-one is formed by dissolution in trifluoroacetic anhydride.[361] Aminolysis of racemic 4-t-butyl 2-phenyloxazol-5(4H)-ones by L-proline . methyl ester leads to almost 100% N-benzoyl-D-t-leucyl-L-proline methyl ester.[362] This is an amazing result, achieved for reactions in aromatic hydrocarbon solvents (results are worse in other solvents and worst in DMF), based on previously-established modest levels of asymmetric induction in this reaction with other hindered oxazolones.

N-Protected α-amino-aldehydes (for a review see ref. 363) are becoming increasingly important among carboxyl-modified amino acids. Their uses in synthesis of ω-amino acids have been discussed earlier (Section 4.15) and further applications in asymmetric synthesis include diastereoselective (syn) addition of lithiated heterocycles (LiR + $(PhCH_2)_2NCHR'CHO \rightarrow (PhCH_2)_2NCHR'CH(OH)R$),[364] of allylsilanes catalyzed by $TiCl_4$ ($ZNHCHRCHO \rightarrow ZNHCHRCH(OH)CH_2CH=CH_2$) with diastereoselectivity dramatically dependent on the relative amount of catalyst,[365] and hetero-Diels-Alder addition to Brassard's diene [$CH_2=C(OMe)CH=C(OMe)OTMS$] to give chiral δ-lactones.[366]

Literature of α-amino acid esters continues to provide a range of routine and novel chemistry. (2-Phenyl-3-butenyl) esters, prepared by coupling the alcohol to the N-protected amino acids using phosgene, are cleaved by ozonolysis. It is difficult to see benefits, and disadvantages appear to be non-crystallinity in representative cases where familiar simple esters are crystalline, as well as the restriction placed on side-chains by the harshness of ester cleavage.[367] Cyanoethyl esters, used for phosphate protection in oligonucleotide synthesis (deblocking involves K_2CO_3 in aq MeOH), have been prepared in the amino acid series using 3-hydroxypropionitrile.[368] 6-Chloro-2-



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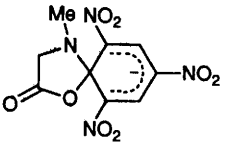

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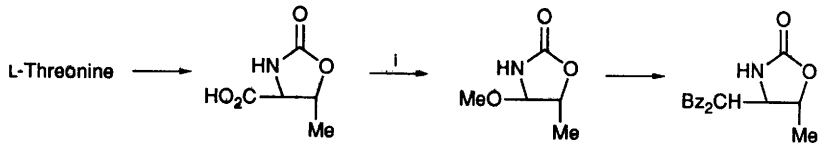
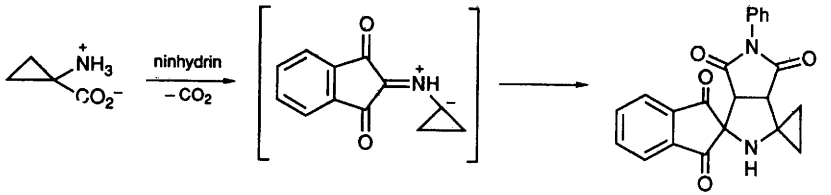


Reagents: i, e^-, MeOH-NaOAc; ii, Bz_2CH_2-TFA

Scheme 38



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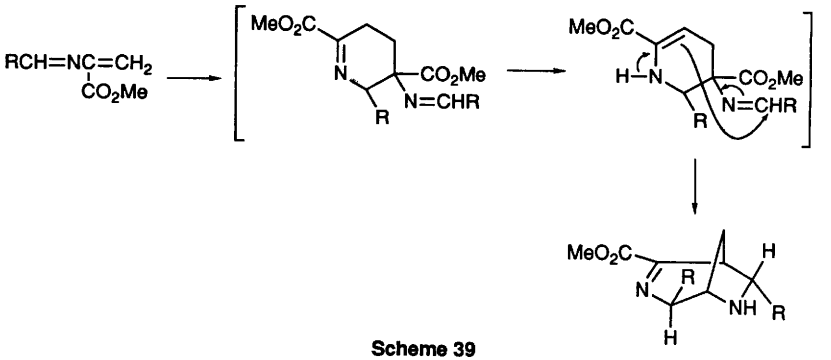


Scheme 39

pyridyl esters show higher reactivity as "active esters" in peptide synthesis than 2-pyridyl esters themselves, and higher than p-nitrophenyl esters, particularly in coupling of Z-Asp(BZL)OR with amino acid esters, there is no detectable aspartimide side-product formed.[369]

p-Chlorotetrafluorophenyl esters, also advocated as "active esters", crystallise better than 2,3,5,6-tetrafluorophenyl analogues, and have higher m.p.s than pentafluorophenyl esters,[370] for which a synthesis using di(pentafluorophenyl)carbonate has been proposed.[371] 9-Fluorenyl esters, formed using diazofluorene[372] and removable by mild acidolysis or hydrogenolysis, are becoming popular for C-protection of amino acids. t-Butyl esters are conveniently prepared using t-butyl fluorocarbonate (Boc-F),[373] better yields being obtainable with this reagent than with $(Boc)_2O$, proposed earlier.[374] A satisfactory protocol for side-chain esterification of Boc-L-aspartic and glutamic α-benzyl esters has been described.[375] Preparations of Z-tyrosine methyl and ethyl esters and hydrazides have been optimized,[376] More controversial is the claim for the formation of Z-amino acid N-acyl-NN-dicyclohexylureas[377] but with physical characteristics different from those recorded by some of us[378] that agree with data described in the literature.[379] Other simple results involving the carboxy group alone (preparation of Boc-L-alanine and glycine N-methylthioamides;[380] and formation of amino acid tetra-n-butylammonium salts,[381]) include in the last-mentioned item a very useful, stable, highly nucleophilic form of amino acids (but not for aspartic and glutamic side-chain esters).

The burgeoning field of "peptide surrogates" requires α-diketone and α-keto-ester homologues of N-protected amino acids, and these are prepared from N-protected α-amino acids *via* N-methoxy-N-methylamides ($RNHCHR'CO_2H \rightarrow RNHCHR'CON(OMe)Me \rightarrow RNHCHR'COC(=CH_2)OEt$ using $CH_2=CHOEt$ with tBuLi, followed by $O_3 \rightarrow RNHCHR'COCO_2Et$ or HCl → RNHCHR'COCOMe).[382]

Substitution at the α-carbon chiral centre in α-amino acids continues to be represented by two main strands: chloride ion-mediated electrochemical methoxylation (see also Section 4.7)[383] and illustrated in Scheme 38 for carboxy-group substitution,[384] and free-radical t-butoxylation (using di-t-butyl peroxide) or halogenation (using N-bromosuccinimide; $PhCONHCHRCO_2Me \rightarrow PhCONHCHRBrCO_2Me$).[385]

A vast volume of data is the only phrase suitable for describing what has accumulated in recent years from studies of enantioselectively-catalyzed hydrolysis of esters of N-acylamino acids. The enzyme-catalyzed aspect of this has been a subject of interest for many years, and recent accounts deal with papain immobilized on Sephadex G-50,[386] or α-chymotrypsin immobilized on poly(vinyl alcohol) by absorption from aqueous solution.[387] These enzymes, and porcine pancreatic lipase, can

cleave allyl esters, even though these are not natural substrates; and there is therefore an alternative to the palladium(0)-catalyzed hydrolysis of these compounds.[388] Acylase I catalyzes the enantioselective hydrolysis of esters of unnatural or rarely-occurring amino acids in the form of their N-acyl derivatives.[389] The potential of this discovery has been realized in the resolution of several examples, including α-methyl-α-amino acids, on a 2 - 29 g scale. Uses for homochiral unnatural amino acids are demonstrated in this study, for example, α-aminobutyric acid enantiomers used for the preparation of (R)- and (S)-1-butene oxide [AcNH- → -Cl → β-chloroalkanols that yield epoxides with KOH], and enantiomers of 2-acetylamino-4-alkenoic acids undergoing iodolactonization to give homochiral 2-acetylamino-4-substituted γ-butyrolactones. The reverse process, use of papain for enantioselective esterification of Boc-amino acids by alkanols and diols, also illustrates the benefits of immobilization; in this report, entrapment in XAD-7 or Sepharose was employed.[390] The other approach, use of synthetic peptides as catalysts for enantioselective ester hydrolysis, also contributes increasingly to the literature, and has been reviewed.[391] N-Acetyl-DL-amino acid p-nitrophenyl esters undergo highly efficient enantioselective hydrolysis (L:D = 167 ± 21) in NN-ditetradecyl-NN-dimethylammonium hydroxide bi-layers through catalysis by N-benzyloxycarbonyl-L-leucyl-L-histidine.[392] Chirality in the quaternary alkyl ammonium hydroxide seems to enhance the stereoselectivity, judging by results for the same system but using NN-ditetradecyl-N-methyl-N-[$CH(OH)_4]CH_2OH^+$ Br^- derived from N-methyl D-glucosamine.[393] Copolymers of N-methacryloyl-L-histidine methyl ester accomplish stereoselective hydrolysis of Z-DL-amino acid p-nitrophenyl esters in the presence of a quaternary alkylammonium hydroxide,[394] and also the analogous methanolysis.[395] To put matters in perspective, a figure should be quoted: 60% optical purity is claimed for Z-L-phenylalanine methylamide formed through hydrolysis of the corresponding DL-p-nitrophenyl ester in the presence of a cationic surfactant (Span 60) and the chiral dioxopiperazine cyclo(L-phenylalanyl-L-histidyl), implying that the D-enantiomer is preferentially hydrolyzed.[396] All permutations of the ingredients just described, are completed with incorporation of the hydrophobic moiety in the substrate, as in N-dodecanoyl-DL-phenylalanine p-nitrophenyl ester + Z-L-Phe-L-His-OMe,[397] or Z-L-Phe-L-His-L-Leu-OH,[398] or in the chiral catalyst, as in N-dodecanoyl-L-histidine, for example,[399] or N-dodecanoyl-L-cysteinamide and the L-histidine and L-histidyl-L-cysteine analogues.[400] The ligand exchange principle has been exploited in this topic area, as exemplified by enantioselective hydrolysis, followed by

potentiometric and spectrophotometric methods, of α-amino acid esters catalyzed by the glycyl-L-tyrosine - copper(II) complex.[401]

6.3 Specific Reactions of Amino Acids.- This section covers papers that describe reactions of side-chains first and foremost, though the amino, carboxy, and α-chiral centre combination (that forms the raison d'etre of the preceding sub-section of this Chapter) may also be involved.

Nearly all the papers covered here, deal with protein amino acids and other familiar biologically-important α-amino acids. Free-radical carboxylation of N^{α}-acetylglycine ethylamide has been studied as a route to aminomalonic acid, a recently-discovered protein amino acid whose presence in proteins may be implicated in pathological calcification of arterial proteins in atherosclerosis.[402] A prior study of the decarboxylation of N^{α}-acetylamino malonic acid ethylamide was carried out to establish the behaviour of the product intended to be formed by carboxylation of glycine derivatives.[403] N-Bromosuccinimide bromination of N-phthaloyl valine and N-phthaloyl phenylalanine occurs at the β-carbon atom, reflecting a steric influence on the course of the reaction, since α-halogenation is more usual in this process (e.g. Ref. 45).[404] Decarboxylation of 1-aminocyclopropanecarboxylic acid and derivatives by ninhydrin provides a special test case for the azomethine ylide intermediate that would be expected (based on recent work discussed in Section 6.2), and indeed found since the cycloadduct (63) is formed with N-phenyl maleimide.[405] Perhaps surprisingly, no ring-opening rearrangement was observed in the preceding example, in spite of the vigorous reaction conditions, but was observed in homolytic side-chain decarboxylation of α-methyl N-Boc-3-cyclopropylglutamate [→ BocNHCH(CO2Me)CEt=CH2].[406] The 3-fluoroglutamic acid analogue underwent clean decarboxylation under the same conditions.

A 93.1% yield of crystalline L-glutamic acid is claimed for the Zn-mediated hydrolytic ring-opening of L-pyroglutamic acid.[407] The precipitated chelate is dissolved in mineral acid, and the solution is brought to the isoelectric point, to complete the surprisingly easy reaction. Selective reduction of Z-aspartic and Z-glutamic anhydrides has been observed with sodium borohydride, leading to α-alkanols.[408]

Reactions of α-hydroxyalkyl-α-amino acids are represented by a preparation of O-benzyl-L-serine that is effective when transient N-(4-methoxybenzyl)oxycarbonylation is employed.[409] A mixed O-seryl O-threonyl O-benzyl phosphate has been synthesized through routine reactions, as a model for the phosphodi-ester linkage in Azotobacter

flavodoxins.[410] Like many other examples of permanganate oxidation, autocatalysis by Mn^{2+} has been established for the oxidation of L-serine.[411] A full paper on the synthesis and nucleophilic ring-opening reactivity of β-lactones from L-threonine and related β-hydroxy α-amino acids has been published.[412] For the preparation of the threonine β-lactone, N-benzenesulphonylthreonine was treated with *p*-bromobenzenesulphonyl chloride and pyridine, since Mitsunobu conditions that were successful with serine gave only the products of decarboxylative anti-elimination with threonine derivatives. There is a tendency for nucleophiles to attack at the carbonyl group rather than at the β-carbon atom (the latter process would be much more useful for general organic synthesis). L-Homoserine can be cyclized *via* its O-trimethylsilyl ester enolate $PhthNC(CH_2CH_2OMEM)=C(OBzl)OTMS$ through catalysis by $TiCl_4$, presumably involving an oxonium chloride intermediate, to give 3-aminotetrahydrofuran-3-carboxylic acid in a novel Mukaiyama aldol condensation.[413]

Formation of (Z)-β-arylamino dehydroalanines $PhCONHC(CO_2Me)=CHNHAr$ from (Z)-dimethylamino- analogues has been demonstrated.[414] The propensity for amino acids with a good leaving group in the γ-position has been considered to be a possible source of toxic vinyl glyoxylates (2-oxo-3-butenoates) *via* unsaturated amino acids.[415] Siting of another unsaturated feature in conjugation with that of the side-chain, as in dehydroalanine Schiff bases $RCH=NC(CO_2Me)=CH_2$, opens up the possibility of Diels-Alder dimerization to 3,4,5,6-tetrahydropyridines. These undergo intramolecular cyclization after tautomerization (Scheme 39).[416]

Lysine reacts with formaldehyde and H_2O_2 to give N-formyl derivatives rather than methylation that is the well-known result of the reaction in the absence of peroxide. The formation of a formaldehyde radical and singlet oxygen is suggested.[417] Monoglycosylated lysine undergoes Amadori rearrangement to give eventually, 4-, 5- and 6-membered nitrogen heterocycles, while diglycosylated lysines lead to pyrans and sugar degradation products.[418] Lysine plays a role in creating crosslinking amino acid residues, *e.g.* allysine, in proteins *in vivo*, another manifestation of the amino group - carbonyl group repertoire of reactions that underlie the two preceding citations. Phthaloyl allysine *p*-nitrobenzyl ester has been synthesized and its reactions with nucleophiles studied to clarify the potential of crosslinks to undergo subsequent changes.[419]

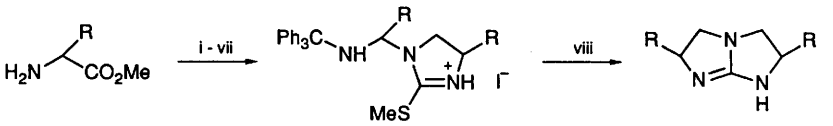
Cysteine is also a versatile performer when presented with other reactive species, and more than 45 compounds are formed with 2,4-decadienal (the major degradation product of linoleic acid) in water during 1 hour at 180°C, 2,4,6-trimethylperhydro-1,3,5-dithiazine being

the major product.[420] Even under controlled conditions, the kinetics of copper- or iron-catalyzed oxidation of cysteine by O_2 are impossible to relate to a mechanistic scheme.[421] More straightforward is the acylation - cyclization of cysteine with 1,1'-carbonyldi-imidazole to give L-2-oxothiazoline-4-carboxylic acid,[422] and S-nitrosation by alkyl nitrites[423] or by N-methyl-N-nitrosotoluene-p-sulphonamide has been clarified to reveal direct nitroso-group transfer.[423] Cysteine or methionine inhibit the nitrosation by nitrous acid, of N-methylaniliine or morpholine under physiological conditions at pH 2.[424] As with other reactive side-chain functionalities, SH-protection is essential for most synthetic applications of cysteine, and reactions of N^{α}-Boc-S-[(N'-methyl-N'-phenylcarbamoyl)sulphenyl]-L-cysteine have been investigated from this point of view.[425] Careful study has been made of iodoacetic acid-mediated hydrolysis of L-methionine to L-homoserine.[426]

The use of abundant chiral α-amino acids in the asymmetric synthesis of target molecules, many of which are of structural types totally unrelated to the amino acids, continues to expand. Stereoselective synthesis of syn-β-amino alkanols from natural amino acids by Reetz and co-workers has been reviewed.[427] D-(-)-Phenylglycine methyl ester has served in a synthesis of chiral guanidines (Scheme 40).[428] (S)-Pyroglutamic acid has been modified (through reduction of its unsaturated functional groups and Mitsunobu substitution) for syntheses in the pyrrolidine series.[429]

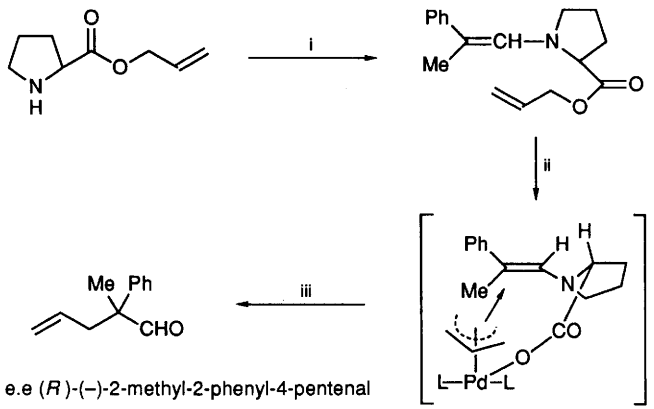
Asymmetric allylation of aldehydes has been elegantly acheived (Scheme 41) using L-proline as chiral determinant.[430] The same imino acid has been used for construction of chiral fused isoxazines.[431] Reactions at aromatic sites in side-chains continiue to be studied from the points of view, of preparative opportunities leading to analogues of common amino acids, and of mechanistic interest. Rate constants for sequential mono- and di-iodination of tyrosine have been elucidated with the help of ^{3}H- and ^{14}C-labelling.[432] Hydroxylation of phenylalanyl and tyrosyl side-chains is the predominant structural change accompanying γ-irradiation of aqueous solutions,[433] and the products of further oxidation, e.g. dopaquinone, can undergo condensation, though the cyclization of this particular compound is inhibited by copper(II) ions.[434] A novel violet colour reaction (λ_{max} 560 nm), shown for tyrosine methyl ester with iron(III) at pH 8, may be useful in analysis. It is suggested to be a 2:1-complex.[435]

Heteroaromatic residues reported to be substituted, include protected tryptophans with benzenesulphenyl or benzeneselenenyl chloride, the final destination of the substituent being the indole 2-position (possibly via rearrangement of an initial 3-substituted isomer).[436]



Reagents: i, Ph_3CCl, NEt_3; ii, $-CO_2Me \longrightarrow CH_2NH_2$; iii, (*R*)-ZNHCHR$CO_2H$, DCC-HOBt; iv, H_2-Pd/C; v, DIBAL; vi, $Cl_2C{=}S$; vii, MeI; viii, DMF/120°C, 1 hour

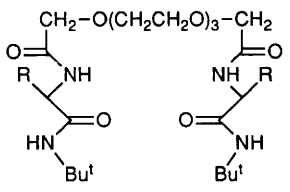
Scheme 40



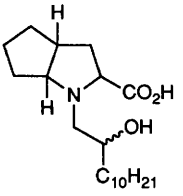
76% e.e (*R*)-(−)-2-methyl-2-phenyl-4-pentenal

Reagents: i, PhCHMeCHO; ii, $Pd(PPh_3)_4$, PPh_3, reflux THF; iii, 10% aq.HCl, reflux 4h

Scheme 41



(64)



(65)

Selective protection problems for piperazine 2-carboxylic acid have been solved by establishing a method for placing a Boc group on one nitrogen function and a Z group on the other.[437] A review of the chemistry of the histidine side-chain has appeared.[438]

Ovothiol A, a natural α-amino acid carrying the mercaptoimidazole functional group in its side-chain, is a more efficient tyrosyl radical scavenger than cysteine or glutathione.[439]

6.4 Effects of Electromagnetic Radiation on Amino Acids.- A number of papers discussed here are concerned with chemical structural changes undergone by photo-excited aromatic and heteroaromatic amino acids, extending studies that have been pursued over many years. Other papers deal with the fundamental physical processes that precede these chemical changes.

Photolysis of aqueous L-tryptophan in the well-known way, to give kynurenine and its N-formyl derivative, occurs without notable extra assistance from uranyl sulphate, compared with other previously-studied photosensitising species.[440]

Photo-CIDNP analysis of those amino acids that are photopolarizable in the presence of a flavin dye (i.e., tyrosine, tryptophan, histidine, N-methylated lysines and methionine) reveal a hydrogen-atom abstraction mechanism for generating radical pairs in the cases of tyrosine and histidine, but an electron transfer mechanism for the other amino acids.[441] A pulsed radiolysis study yields redox midpoint potentials for a neutral radical created in the indole moiety of the tryptophan side-chain.[442]

Effects of solvent on fluorescence characteristics of tyrosine and its derivatives have been elucidated.[443] Single-photon timing data have been extracted from fluorescence decay curves measured for tryptophan solutions at pH 6 for various emission wavelengths.[444] Solvent complexation of tryptophan has been studied by laser-induced fluorescence excitation spectroscopy of solutions subjected to supersonic free-jet expansion.[445]

7 Analytical Methods

7.1 General.- Early Chapters in a recent book on protein analysis, review current methods for amino acid analysis.[446] The general situation amounts to the consolidation of existing methods, with some success at raising sensitivity levels, and substantial progress with determinations of enantiomeric composition.

7.2 Gas-Liquid Chromatography.- Derivatization protocols continue to favour N-pentafluoropropionyl[447] and heptafluorobutyroyl butyl esters,[448,449] though N,O(S)-t-butyldimethylsilyl derivatives continue to hold their initial promise.[450] These studies, representative of a larger routine literature, have been selected to illustrate different detection methods (FTIR; ECD; CIMS), and other aspects: "losses" that occur during derivatization, and other problems of interference; trace analysis (amino acids in 600My sedimentary rocks; GABA and Glu in rat brain striatal microdialyzate at 6 pg sensitivity, 20μL fluid containing 105.5 pg GABA and 9.4ng Glu).

A stationary phase carrying the N,N'-3,6,9-oxadecanoyl-bridged L-phenylalanine t-butylamides (64) has been proposed for analytical resolution of amino acids by capillary g.l.c. of the N-trifluoroacetyl derivatives of their alkyl esters.[451] An n.m.r.study of the mechanism of chiral recognition by this host in solutions containing amino acid derivatives as guest, has been included in this paper. An application of this system for analysis of the D-amino acid composition of foods has been published.[452] A Chirasil-type chiral polysiloxane di-amide has been investigated for enantiomeric analysis by g.l.c.[453]

7.3 Ion-Exchange Chromatography.- There is some overlap with the later h.p.l.c. section, as far as trace analysis is concerned. Non-routine papers include amino acids in fossil bones (samples available only at the milligram level),[454] a study of equilibria and rates of uptake of phenylalanine and tyrosine by the strong acid cation exchange resin, Amberlite 252,[455] and estimation of total cysteine + cystine in proteins after reduction and derivatization with 3,3'-dithiopropionic acid,[456] and of homocysteine in plasma (elution with dithreitol-containing buffers).[457]

Sorption of amino acids on to a cellulose-based ampholyte represents an ongoing study of potential new ion-exchange media.[458]

7.4 Thin Layer Chromatography.- As with a number of other topic areas, techniques that have become thoroughly routine and therefore generate literature that is largely excluded from this Chapter are given an apparently uneven airing for this reason.

In the "overpressured" mode (one mobile phase moving over another immiscible liquid) the simultaneous t.l.c. of 100 or more plates has been demonstrated for PTHs.[459] Further data enabling the parametrization of 55 amino acids for peptide QSAR (see Vol.21, p.55) include t.l.c. in several systems.[460] Several papers from one research group constitute a concise picture of current uses of t.l.c. for enantiomeric analysis.

The topic has been reviewed in general terms[461] and specifically (for post-1972 literature) for dansyl and 2,4-dinitrophenylamino acids.[462] The copper(II) complex of the D-proline-derived species (65) has been used to impregnate reversed-phase octadecylsilica t.l.c. plates for the purpose of enantiomer separation of imino acids.[463]

7.5 High Performance Liquid Chromatography.- In the context of this Chapter, the literature on this technique cannot yet be said to be recycling established results (as it appears to be in some other analytical areas). H.p.l.c. offers a less expensive amino acid analysis facility than any other instrumental technique, with the opportunity to use the h.p.l.c. instrumentation for other analytical purposes. What is needed - and not exactly lacking - is a clear statement of what is the best chemistry for the purpose, since there are many derivatization protocols available. Appraisal of four reagents, o-phthaldialdehyde, phenyl isothiocyanate, Fmoc chloride, and dansyl chloride,[464] for the analysis of 24 - 26 of the major amino acids in 20 - 30 minutes leads to the brief judgments, that phenyl isothiocyanate is the least sensitive, and its reproducibility and linearity are poor (and identification of cysteine is not possible), but the other three methods are reliable. A similar review of fluorescence-based methods concludes that the o-phthaldialdehyde method is most suitable compared with fluoresceamine and 7-chloro-4-nitrobenzofurazan (but the latter method is resorted to for proline and other imino acids since the o-phthaldialdehyde method is not applicable to these).[465] A comparison of h.p.l.c. with the revived methods of electrophoresis has been reviewed.[466]

A number of fundamental physical studies are included in the recent literature, including the influence of salts on the retention of amino acid derivatives on reversed phase columns,[467] adsorption of amino acids on to spherical titania [amorphous titanium(IV) hydrous oxide],[468] and on to synthetic hydroxylapatite.[469] Microbore h.p.l.c. has been authoritatively reviewed.[470]

Use of h.p.l.c. for the detection of N-nitroso-imino acids has been described.[471] Another class of "naturally-derivatized amino acids", as they might be called, that is represented in the h.p.l.c. literature, is the unusual but not uncommon α-amino acid amide group. Many important peptides possess an amidated C-terminus, which might be released intact in biological breakdown processes. Thermospray mass spectrometry detection in biological samples, down to 1 pmol levels, with ammonium formate added post-column to generate the necessary ions in the m.s. ion source, has been explored.[472]

Phenyl isothiocyanate dervatization has a major role now, in h.p.l.c. analysis of amino acids. Papers offering creative contributions include thermospray m.s. detection,[473] application to identification of amino acid hydrazide mixtures formed by hydrazinolysis of peptides, and to identify the C-terminal amino acid - the only residue not to appear as an amino acid hydrazide in the hydrazinolysate,[474] attention to composition of solvents for gradient elution,[475,476] and sources of contamination.[477] Several articles on PTC-amino acid technique, appear in this source,[477] e.g. discussing the Applied Biosystems automated system.[478] Two new isothiocyanate reagents have been described, that incorporate a ferrocenyl moiety as "electrophore" - i.e. sensitive to electrochemical detection.[479]

4-Substituted N-phenylthiohydantoins (PTHs) continue to be reported upon for their h.p.l.c. properties. The purpose of these studies is to improve the analytical aspect that is on the end of the Edman peptide sequencing chemistry; an auto-injection system has been described, that is not dependent on critical timing for efficient transfer of PTHs from the conversion flsk to the sample loop of the injection valve.[480] Picomole analysis of PTHs, using the BAS 200A instrument,[481] and sub-picomole analysis,[482] and other details in particular applications,[483] have been described.

The o-phthaldialdehyde - thiol reagent system continues to be the mainstay for h.p.l.c. amino acid analysis, though awareness of its potential for erratic results must be well known to all users (cf. Refs.347 and 348). An elegant study using ^{14}C-labelled amino acids shows that the 2-alkythio-isoindoles created by the OPA - thiol reagent are unstable during h.p.l.c. on a reverse phase C-18 column.[484] Comparison of the emergence profile as determined by radioactivity, with the fluorescence detection profile, shows that there is excessive "^{14}C-fronting" of the peaks. This is accounted for on the basis of faster-running ^{14}C-containing degradation products. Column half-lives of glutamic acid, arginine, and ornithine iso-indoles are 16, 40, and 54 minutes respectively, explaining the importance emphasised in several papers, of following a strict protocol so that reliable results might be obtained using the OPA method. The same ^{14}C-monitoring method applied to PTC-amino acids showed superimposed ^{14}C- and light absorption profiles, indicating no degradation of PTC-derivatives on the h.p.l.c. timescale. Not only is the iso-indole product unstable, but the premixed reagent system would also be expected to be unstable (as a result of thiol oxidation), and this has now been shown to be so,[485] but this uncertainty factor, once appreciated, can be eliminated by proper attention to operating technique.

As part of a study of the proteolytic activity of Lactobacillus bulgaricus, a comparison of the OPA technique with classical ion-exchange analysis puts them at equal ranking, but notes that the ion-exchange methods provide additional analytical information.[486] A number of papers have appeared employing the OPA technique in different contexts: neurotransmitter amino acids (glutamic acid at 0.5 pmol level, and aspartic acid, in rat striatum using electrochemical detection,[487] corresponding results for the same method, using 2-methylpropane-2-thiol but detecting at 50-100 fmol levels,[488] and using a broader range (18 represented) of amino acids).[489] OPA - mercaptoethanol has been used for the analysis of amino acids in tea,[490] in blood samples,[491] and in exploratory studies of automated operation.[492] One bonus of the OPA - thiol reagent system is the opportunity to use a chiral thiol so that proportions of enantiomers in derivatized amino acids can be determined since they lead to diastereoisomer mixtures. OPA - N-acetyl-L-cysteine continues to be used reliably in this context. This study includes a comparison of this method with the "chiral mobile phase" approach using copper(II) acetate and L-proline in isocratic h.p.l.c. analysis of representative α-amino acids and their α-alkyl analogues.[493]

A new variant is the use of naphthalenedialdehyde with CN^-, yielding fluorescent cyano[f]benzoiso-indoles with amino acids, that are more stable than the OPA - thiol adducts, and suitable for electrochemical detection.[494] The potential of the method is illustrated with assays of desmosine and isodesmosine protein cross-linking amino acid residues in proteins.

9-Fluorenylmethoxycarbonyl chloride (Fmoc-Cl) is making headway in competition with the other fluorescent-derivatization protocols, and its complementary nature with the OPA method has been pointed out;[495] a commercial robotic autosampler can effect OPA adduct formation (amino acids) followed by Fmoc-Cl derivatization (imino acids), and then h.p.l.c. The accuracy of the Fmoc protocol has been compared favourably with that of the other standard methods,[496] and shown to be routinely convenient.[497] The supercritical fluid chromatography of Fmoc-amino acids and of the diastereoisomer mixtures formed with the chiral analogue (+)-1-(9-fluorenyl)ethyloxycarbonyl chloride ("Flec-Cl") have been emphatically established, opening up exciting new possibilities.[498]

Several other fluorescence-generating derivatization reactions have been developed, monobromobimane for homocysteine h.p.l.c.,[499] naphthyl isocyanate,[500] and 4-(N-1-dimethylaminonaphthalene-5-sulphonylamino)phenyl isothiocyanate, an ingenious combination of the dansyl and PTC-derivatives.[501] Preparation of DABS-amino acids using 4'-

(NN-dimethylaminoazobenzene)sulphonyl chloride (dansyl chloride) and their derived DABTH's for h.p.l.c. analysis of amino acids[502] is continuing to gain advocates.[503] Better recovery of serine and threonine derivatives is claimed for preparations of DABTH's using BF_3 etherate in place of trifluoroacetic acid for the conversion step for substituted anilinothiazol-5(4H)-ones created in amino acid sequence analysis. These derivatives allow detection down to 100 fmol levels.[504] An ingenious alterative to converting such thiazolones into thiohydantoins is to aminolyse them with 4-aminofluorescein, to give PTC-amino acid fluoresceinamides; these can be detected down to 100 amol levels.[505] Similar h.p.l.c. studies of fluorescent 4-(NN-dimethylamino)-1-naphthyl thiohydantoins[506] has been described.

Miscellaneous h.p.l.c. studies extending the specific examples described above, include the six putative neurotransmitter amino acids (h.p.l.c. analysis after derivatization with electrochemical detection),[507] amino acids in cerebrospinal fluid,[508] and six coded amino acids in dried blood samples.[509]

Chiral h.p.l.c. of amino acid derivatives using either chiral stationary phases based on Boc-D-valine bonded to silica[510] or Pirkle-type phases with (R)-N-(3,5-dinitrobenzoyl)phenylglycine bonded to γ-aminopropyl-silica[511] (as used for the resolution of N^{α}-substituted DL-amino acid anilides).[512] α-Chymotrypsin bonded to silica gel gives a chiral phase that has been used for the resolution of amino acid esters - though not only through the familiar enantioselectivity of catalyzed hydrolysis but using the enzyme as a chiral surface, since the inhibited enzyme was also effective in h.p.l.c. resolution.[513] Chiral mobile phases incorporating copper(II) complexes of α-t-butyl L-aspartate,[514] proline,[515] and ligands of the di-amino di-amido-type containing L-amino acid moieties[516] have been used for amino acid analytical resolution (for dansyl DL-amino acids in the last-mentioned example).

7.6 Fluorescence Analysis.- This section is reserved for methods that appear promising or have become established, for fluorescence-generating reactions that do not depend on subsequent h.p.l.c. procedures. Thus, post-column OPA - 3-mercaptopropanoic acid fluorimetry allows estimation of amino acids separated by ion exchange chromatography with sensitivity at low picomole levels,[517] and N-(2-pyridyl)amino acids formed by condensation of amino acids with 2-aminopyridine, might show similar potential.[518]

The authors' 1979 method for tryptophan estimation in food, based on measuring the fluorescence increase following N^{in}-hydroxymethylation

caused by addition of formaldehyde at pH 10, has been given further attention through assessing the pH-dependence of the fluorescence.[819]

7.7 Other Analytical Methods.- Capillary zone electrophoresis seems destined to take a larger role, with further impressive results such as sub-attomole detection of fluorescein isothiocyanate adducts using laser-induced fluorescence measurements.[820] Following polyacrylamide-gel electrophoresis of amino acids and peptides in an acetic acid - formic acid pH 2 buffer, using fabric-reinforced gels, the samples were fixed by freeze-drying of excised zones. The purpose was to be able to perfuse the zones uniformly with ninhydrin solution, so as to give lowered detection limits, and 0.1 - 0.25 μg levels were achieved.[821] Micellar electrokinetic chromatography on chiral media has been demonstrated as capable of the resolution of dansyl-DL-amino acids.[822] Specific chemiluminescence of excited NO_2, created using a commercial nitrogen analyzer in which amino acids are combusted at 1000 - 1100°C to give NO, converted by ozone oxidation into the excited NO_2, can be detected with 200 times greater sensitivity than existing (Lowry) nitrogen oxides analysis and therefore operates at sub-microgram sample levels.[823]

7.8 Determination of Specific Amino Acids.- Molecular structural factors from which specific assays can be derived occur in histidine and in tyrosine. Tyrosine PTH, or the amino acid itself, or histidine - copper(II) complex coupled with diazotized sulphanilic acid, can be assayed by adsorptive stripping voltammetry at low levels.[824] Characteristic polarographic profiles have been determined for histidine.[825]

This section continues to report enzyme electrodes coping with tyrosine, DOPA, and α-methylDOPA, based on tyrosinase immobilized on a pH electrode,[826] and an electrode for L-asparagine using a sensor employing L-aspartase immobilized on an ammonia-sensing probe (capable of 1.6×10^{-5} - 1.5×10^{-3} molar sensitivity, and stable for more than 30 days),[827] and a more horticultural version (presumably with a shorter lifespan!) of the same system using minced parsley leaves on a potentiometric ammonia gas sensor, for L-asparagine and L-glutamine assays.[828]

8 References

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