1
Amino Acids

BY G. C. BARRETT

1 Introduction

Thorough coverage centred on the 1984 literature, though omitting routine biological applications and reports of the distribution of well-known amino acids, is the intention for this chapter. There is therefore continuity with preceding volumes of this *Specialist Periodical Report* (to which reference is occasionally made in order to help the reader put into context some recent progress reported here for an on-going topic of study).

2 Textbooks and Reviews

The 1983 recommendations for amino acid nomenclature are only a library distant, since the I.U.P.A.C.-I.U.B. Newsletter (1984) has been reproduced in major journals.[1a] The recommendations have been quickly followed by nomenclature for amino acid amides (1984).[1b]

Important compilations providing support of research work with amino acids represent the latest outputs from sources already well known for similar recent monographs.[2] Other reviews, much less readily accessible, deal with various facets of medium- to large-scale production of amino acids.[3]

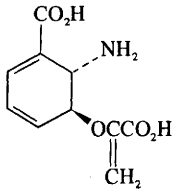
[1] (*a*) 'Nomenclature and Symbolism for Amino Acids and Peptides (Recommendations 1983)', *Eur. J. Biochem.*, 1984, **138**, 9; *Arch. Biochem. Biophys.*, 1984, **229**, 399; *Int. J. Pept. Protein Res.*, 1984, **24** (1); *Hoppe-Seyler's Z. Physiol. Chem.*, 1984, **365**, 1; *Can. J. Biochem. Cell Biol.*, 1984, **62**, viii; *Pure Appl. Chem.*, 1984, **56**, 595; *J. Biol. Chem.*, 1985, **260**, 14; (*b*) *Biochem. J.*, 1985, **225**, 1.

[2] *Methods Enzymol.*, 1984, **106**: H. L. Cooper, M. H. Park, and J. E. Folk, p. 344 (hypusine), R. L. Sass and M. E. Marsh, p. 351 (N^{π}- and N^{τ}-histidinoalanine), A. N. Glazer, p. 359 [S^{β}-(bilin)cysteine derivatives], K. Lerch, p. 355 [S^{β}-(2-histidyl)cystine]; *Methods Enzymol.*, 1984, **107**: R. Amado, R. Aeschbach, and H. Neukom, p. 377 (dityrosine), S. C. Fry, p. 388 (iodotyrosine), H. J. Waite and C. V. Benedict, p. 397 (dopa), S. Hunt, p. 413 (halogenated tyrosines), G. L. Nelsestuen, p. 503 (γ-carboxyglutamic acid), T. H. Koch, M. R. Christy, R. M. Barkley, R. Sluski, D. Bohemier, J. J. Van Buskirk, and W. M. Kirsch, p. 563 (synthesis of β-carboxyaspartic acid and identification of γ-carboxyglutamic acid), M. X. Sliwkowski, p. 620 (*Se*-adenosyl selenomethionine); 'CRC Handbook of H.P.L.C. for the Separation of Amino-acids, Peptides, and Proteins', ed. W. S. Hancock, Chemical Rubber Company, Boca Raton, Florida, U.S.A., 1984, Vols. 1 and 2 [*e.g.* S. Ishimitsu, S. Fujimoto, and A. Ohara, p. 275 (*o*-, *m*-, and *p*-tyrosine)].

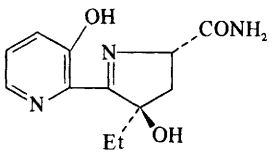
[3] K. Yokozeki, C. Aguchi, and Y. Hirose, *Ann. N.Y. Acad. Sci.*, 1983, **413**, 551; K. Matsumoto and I. Chibata, *Hakko to Kogyo*, 1983, **41**, 834 (*Chem. Abstr.*, 1984, **100**, 175 219); B. Hoppe and J. Martens, *Chem Unserer Zeit*, 1984, **18**, 73; 'Preparation of Optically-active Amino-acids: Theoretical Principles, Problems, Horizons', I. Yu. Galaev and Yu. V. Galaev, *Izd. Sarat. Univ., Saratov, U.S.S.R.*, 1983 (*Chem. Abstr.*, 1984, **101**, 171 747).

3 Naturally Occurring Amino Acids

Occurrence of Known Amino Acids. – Points of interest to appeal to a cross-section of readers are found in the location of D-2-aminopimelic acid and its *trans*-3,4-dehydro analogue in *Asplenium unilaterale*,[4] of five γ-carboxyglutamic acid residues within a novel heptadecapeptide toxin in the venom of a fish-hunting cone snail, *Conus geographus*,[5] and of the crosslinking amino acid residues lysinoalanine (in alkali-treated partial hydrolysates of β-casein and broad-bean protein)[6] and 3-hydroxypyridinium-containing moieties (in cartilage).[7] Other heteroaromatic and aromatic moieties of familiar types feature in a useful study of optimum conditions for protein hydrolysis in which tryptophan degradation is largely avoided (92% recovery using 3M mercaptoethane sulphonic acid at 166 °C for 25 min)[8] and in structure elucidation of the common aglycone moiety of the actaplanin antibiotics (made up of hydroxylated phenylalanine and phenylglycine units condensed into a tetracyclic peptide array).[9]



(1)



(2)

New Natural Amino Acids. – First findings reported in this section range from free amino acids [*trans*-4-hydroxy-*N*-methyl-L-proline in the red alga *Chondria coerulescens*[10] and an intermediate (1) in the transformation of chorismic acid to anthranilic acid by anthranilate synthase I from *Serratia marcescens*][11] to simple derivatives histargin (a new carboxypeptidase B inhibitor from *Streptomyces roseoviridis*, in which arginine and histidine are linked *via* carboxy groups by 1,2-diaminoethane),[12] siderochelin C (2) from an *Actinomycete*,[13] and an

[4] N. Murakami and S. Hatanaka, *Phytochemistry*, 1983, **22**, 2735.
[5] J. M. McIntosh, B. M. Olivera, L. J. Cruz, and W. R. Gray, *J. Biol. Chem.*, 1984, **259**, 14 343.
[6] H. Noetzold, H. Winkler, B. Wiedemann, and E. Ludwig, *Nahrung*, 1984, **28**, 299 (*Chem. Abstr.*, 1984, **101**, 67 984).
[7] J. J. Wu and D. R. Eyre, *Biochemistry*, 1984, **23**, 1850.
[8] K. Maeda, J. J. Scheffler, and A. Tsugita, *Hoppe-Seyler's Z. Physiol. Chem.*, 1984, **365**, 1183.
[9] A. H. Hunt, T. K. Elsey, K. E. Merkel, and M. Debono, *J. Org. Chem.*, 1984, **49**, 641.
[10] S. Sciuto, R. Chillemi, M. Piattelli, and G. Impellizzeri, *Phytochemistry*, 1983, **22**, 2311.
[11] P. P. Policastro, K. G. Au, C. T. Walsh, and G. A. Berchtold, *J. Am. Chem. Soc.*, 1984, **106**, 2443.
[12] H. Umezawa, T. Aoyagi, K. Ogawa, H. Iiunima, H. Naganawa, M. Hamada, and T. Takeuchi, *J. Antibiot.*, 1984, **37**, 1088.
[13] L. A. Mitscher, T. Hogberg, S. D. Drake, A. W. Burgstahler, M. Jackson, B. Lee, R. I. Sheldon, H. E. Gracey, W. Kohl, and R. J. Theriault, *J. Antibiot.*, 1984, **37**, 1260.

unusual deoxynucleotide, α-*N*-(9-β-D-2′-deoxyribofuranosylpurin-6-yl)glycinamide, specified by bacteriophage Mu.[14]

New Amino Acids from Hydrolysates. – This section continues to record unsuspected and unlikely (but real) protein amino acids, with a spectacular 'first', the location of aminomalonic acid, $H_3\overset{+}{N}CH(CO_2^-)CO_2H$, in *Escherichia coli* and atherosclerotic plaque proteins (the latter also contain β-carboxyaspartic and γ-carboxyglutamic acids).[15]

Although both *cis* and *trans* isomers of 3- and 4-hydroxyproline appear in collagen hydrolysates, the *cis* isomers are formed during the hydrolysis procedure.[16]

The presence of ε-(γ-glutamyl)lysine in protein hydrolysates has been established through sensitive h.p.l.c. methods.[17]

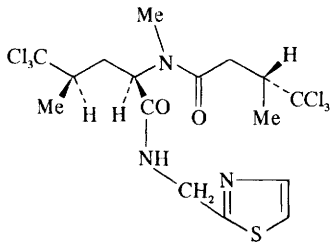
4 Chemical Synthesis and Resolution of Amino Acids

General Methods of Synthesis. – Amination of simple substrates is represented in reactions of sodium chloroacetate with secondary amines in tetrahydrofuran[18] and of aliphatic aldehydes with $CHCl_3$ and NH_3 in CH_2Cl_2–H_2O containing a phase-transfer agent[19] and in reductive amination of keto acids using sodium cyanoborohydride and an ammonium salt.[20] Analogous carboxylation processes are represented in electroreduction of Schiff bases $PhCR^1{=}NCHR^2Ph$ in the presence of CO_2[21] and in hydrocarbonylation of *N*-vinyl- and -allyl-phthalimides catalysed by Rh or Pd complexes.[22]

Standard procedures are employed in alkylation of diethyl acetamidomalonate (*e.g.* 2,6-dihalotyrosines[23]), formation of α-aminonitriles ($RCHO + Me_3SiCN$ catalysed by $ZnI_2 \rightarrow NCCHROSiMe_3$, which is reacted with a secondary amine in MeOH),[24] more conventional Strecker synthesis of alicyclic α-amino acids from corresponding ketones and $PhCH_2NH_2$ with KCN,[25] azlactone syn-

[14] D. Swinton, S. Haltmann, P. F. Crain, C. S. Cheng, D. L. Smith, and J. A. McCloskey, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, **80**, 7400.
[15] J. J. Van Buskirk, W. M. Kirsch, D. L. Kleyer, R. M. Barkley, and T. H. Koch, *Proc. Natl. Acad. Sci. U.S.A.*, 1984, **81**, 722.
[16] G. Bellon, R. Berg, F. Charstang, A. Malgras, and J. P. Borel, *Anal. Biochem.*, 1984, **137**, 151.
[17] M. Griffin and J. Wilson, *Mol. Cell. Biochem.*, 1984, **58**, 37.
[18] F. M'Henni and Z. Mighri, *J. Soc. Chim. Tunis*, 1984, **11**, 3 (*Chem. Abstr.*, 1984, **101**, 171 679).
[19] X. Sun, Y. Shi, H. Zhu, Z. Zhou, and C. Lin, *Nanjing Daxue Xuebao, Ziran Kexue*, 1983, 658 (*Chem. Abstr.*, 1984, **100**, 210 384).
[20] S. P. Reid and P. J. Reeds, *Anal. Biochem.*, 1984, **142**, 24 (*cf.* R. F. Borsch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, 1971, **93**, 2897).
[21] T. Iwasaki and K. Harada, *Bull. Inst. Chem. Res., Kyoto Univ.*, 1983, **61**, 72 (*Chem. Abstr.*, 1984, **100**, 121 561).
[22] G. Delogu, G. Faedda, and S. Gladiali, *J. Organomet. Chem.*, 1984, **268**, 167.
[23] R. A. Pascal and Y. C. J. Chen, *J. Org. Chem.*, 1985, **50**, 408.
[24] K. Mai and G. Patil, *Tetrahedron Lett.*, 1984, **25**, 4583.
[25] W. J. Layton, S. L. Smith, P. A. Crooks, T. Deeks, and R. D. Waigh, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1283.

thesis,[26–28,150] alkylation of isocyanoacetic esters[29] and glycine derivatives [*e.g.* the Schiff base $Ph_2C{=}NCH_2CO_2Et$[30] and $PhSCH_2NMeCH_2CO_2Et$,[31] the latter with NaH undergoing cycloaddition with $PhCH{=}C(CO_2Me)_2$ in HMPA–dimethoxyethane to yield *N*-methylproline derivatives[31]], and an example of the Ugi four-component condensation, leading to compound (3).[32]



(3)

A new synthesis has been reported, based on the rearrangement of acetimidates $R^1CH{=}CHCHR^2OC(=NH)CCl_3$ derived from allylic alcohols.[33,93] Overnight refluxing in xylene followed by treatment of the resulting amide $CCl_3CONHCHR^2CH{=}CHR^1$ with $NaIO_4$–RuO_3 then hydrolysis in aqueous HCl gives the amino acid $H_3\overset{+}{N}CHR^2CO_2^-$. The potential of this method is limited by both the accessibility of the allylic alcohol and the compatibility of the eventual amino acid side chain R^2 with the reaction conditions (the conversion of an alcohol into the acetimidate requires NaH and CCl_3CN as reagents).

Asymmetric Synthesis. – Many of the recent papers on this topic cover what could be described as established general methods, since many of them extend studies that have featured in this section in preceding volumes. One of the longest-established of these, the asymmetric hydrogenation of amino-acrylates,[34]

[26] A. K. Sen and S. Mukhopadhyay, *Indian J. Chem., Sect. B*, 1983, **22**, 939 (*Chem. Abstr.*, 1984, **100**, 210 368).

[27] P. K. Tripathy and A. K. Mukerjee, *Synthesis*, 1984, 418.

[28] M. Ali and N. H. Khan, *Indian J. Chem., Sect. B*, 1984, **23**, 868 (*Chem. Abstr.*, 1984, **101**, 230 986).

[29] C. Herdeis and U. Nagel, *Heterocycles*, 1983, **20**, 2163.

[30] M. J. O'Donnell, W. Bruder, K. Wojciechowski, L. Ghosez, M. Navarro, F. Sainte, and J. P. Antoine in 'Peptides: Structure and Function', Proceedings of the 8th American Peptide Symposium, ed. V. J. Hruby and D. H. Rich, Pierce Chemical Company, Rockford, Illinois, U.S.A., 1983, p. 151.

[31] N. Imai, Y. Terao, K. Achiwa, and M. Sekiya, *Tetrahedron Lett.*, 1984, **25**, 1579.

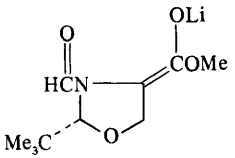
[32] S. E. De Laszlo and P. G. Williard, *J. Am. Chem. Soc.*, 1985, **107**, 199.

[33] S. Takano, M. Akiyama, and K. Ogasawara, *J. Chem. Soc., Chem. Commun.*, 1984, 770.

[34] I. Ojima, *Pure Appl. Chem.*, 1984, **56**, 99; K. Harada and M. Takasaki, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 1427; T. Yamagishi, M. Yatagai, H. Hatakeyama, and M. Hida, *ibid.*, p. 1897; M. Inoue, K. Ohta, N. Ishizuka, and S. Enomoto, *Chem. Pharm. Bull.*, 1983, **31**, 3371; L. O. Nindakova, F. K. Shmidt, E. I. Klabunovskii, and V. A. Pavlov, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1984, 720.

azlactones,[35] and Schiff bases,[36] is represented in familiar forms, employing chiral phosphine–Rh or –Co catalysis[34] or the incorporation of a chiral moiety into the substrate.[34,36] The protonation of amino-acrylic acid itself occurs with modest (15–20%) enantiomeric excess during its conversion into alanine catalysed by the *Pseudomonas striata* amino acid racemase.[37]

The reason for the continuing flow of papers is the incomplete understanding of the relationship between stereoselectivity and structure in this area of asymmetric synthesis. This uncertainty also applies to asymmetric transaminations of α-keto acids using a chiral pyridoxamine[38] and aminolysis of azlactones by chiral amines.[39] The crop of papers in which asymmetric alkylation processes are extended generally describe high stereoselectivity, however; a synthesis of α-methylated (*S*)-amino acid esters $H_2NCRMeCO_2Me$ through alkylation of the Schiff base of methyl L-alaninate with an alkyl bromide RBr after lithiation with $LiNPr^i_2$, where the Schiff base is formed with the aldehyde formed from 1,2,3,4-protected D-galactose (*cf.* precedent work, Vol. 14, p. 11),[40] gives enantiomeric excesses of 44–85%.[40] L-Serine undergoes α-alkylation with retention of its configuration through reaction of the derived lithium enolate (4) with electrophiles.[41] The same principle also applies to the asymmetric alkylation of nickel(II) complexes of (*N*-benzyl-L-prolyl)-*o*-aminobenzaldehyde with acetaldehyde, leading to L-threonine and its *allo* isomer in enantiomeric yields of 86 and 76%, respectively.[42] Amides formed between DL-amino acids and (*S*)-prolinol methyl ether can be lithiated and re-protonated with up to 92% diastereoselectivity;[43] much less selectivity is seen in the alkylation of DL-amino acids esterified either with (*S*)-prolinol or with (–)-menthol, since diastereoisomeric excesses range from 5 to 46%.[43]



(4)

[35] E. I. Karpeiskaya, L. F. Godunova, E. S. Levitina, M. R. Lyubeznova, E. I. Klabunovskii, E. D. Lubuzh, and A. I. Lutsenko, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1984, 85.

[36] K. Harada and S. Shioni, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 1367.

[37] B. Badet, K. Lee, H. G. Floss, and C. T. Walsh, *J. Chem. Soc., Chem. Commun.*, 1984, 838.

[38] Y. Tachibana, M. Ando, and H. Kuzuhara, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 3652; K. Bernauer, R. Deschenaux, and T. Taura, *Helv. Chim. Acta*, 1983, **66**, 2049; R. Deschenaux and K. Bernauer, *ibid.*, 1984, **67**, 373.

[39] L. N. Kaigorodova, E. S. Levitina, E. I. Karpeiskaya, and E. I. Klabunovskii, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1984, 813.

[40] I. Hoppe, U. Schöllkopf, and R. Toelle, *Synthesis*, 1983, 789.

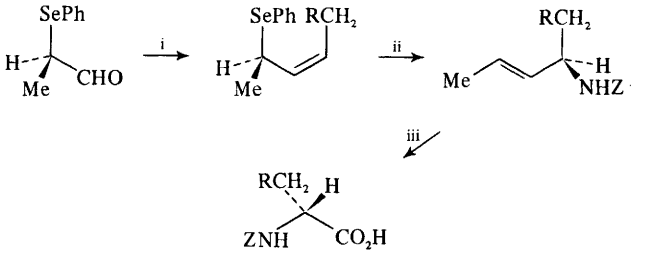
[41] D. Seebach and J. D. Aebi, *Tetrahedron Lett.*, 1984, **25**, 2545.

[42] Yu. N. Belokon, N. I. Chernoglazova, K. A. Kochetkov, N. S. Garbalinskaya, M. G. Ryzhov, V. I. Bakhmutov, M. B. Saporovskaya, E. A. Paskonova, and V. I. Maleev, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1984, 804.

[43] K. G. Davenport, D. T. Mao, C. M. Richmond, D. E. Bergbreiter, and M. Newcomb, *J. Chem. Res. (S)*, 1984, 148.

Continuing studies[44] with alkylation of lithiated 2,5-dimethoxy-3,6-dihydropyrazines (see also refs. 42 and 76) confirm the high levels of enantiomer purity that can be achieved (see Vol. 16, p. 6; for a review see ref. 45). For example,[44] condensation of the (*S*)-3-isopropyl compound with $Me_3CSiMe_2CR^1R^2CHO$ after lithiation gives $R^1CR^2{=}CRCH(\overset{+}{N}H_3)CO_2^-$ in better than 95% enantiomer excess.

A novel transfer-of-chirality operation applied to the synthesis of *N*-benzyloxycarbonyl-D-amino acids in 78–84% enantiomer excess is based on [2,3]-sigmatropic rearrangement of a vinyl selenide (Scheme 1).[46]



Reagents: i, Wittig synthesis; ii, $PhCH_2OCONH_2$; iii, O_3, Jones oxidation

Scheme 1

Prebiotic Synthesis Models. – The ripples continue to spread out from the original 'electric discharge-$CH_4/H_2O/N_2$ or NH_3' experiment, and as in recent years (see Vol. 16, p. 6) one of the original authors has again reappeared on the expanding wavefront with a comparison of relative yields of C_3–C_6 amino acids in such a system when the NH_3 concentration varies and when other simple alkanes are used in place of methane.[47] Shock-wave compression (amplitude 10 GPa) converts ammonium salts of acrylic, crotonic, cinnamic, and fumaric acids into β-alanine, β-aminobutyric acid, phenylalanine, and aspartic acid, respectively, in yields of up to 10%.[48]

Extended reaction times convert glycine-formaldehyde or -acetaldehyde mixtures at pH 3.5 and at 60–80 °C not only into the expected serine and threonine but also into alanine, glutamic acid, aspartic acid, norvaline, isoleucine, and four other protein amino acids.[49]

[44] U. Schöllkopf, J. Nozulak, and U. Groth, *Tetrahedron*, 1984, **40**, 1409; Y. Jiang, U. Groth, and U. Schöllkopf, *Huaxue Xuebao*, 1984, **42**, 86 (*Chem. Abstr.*, 1984, **100**, 210 372).

[45] U. Schöllkopf, *Pure Appl. Chem.*, 1983, **55**, 1799.

[46] J. N. Fitzner, R. G. Shea, J. E. Fankhauser, and P. B. Hopkins, *J. Org. Chem.*, 1985, **50**, 417.

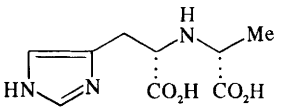
[47] D. Ring and S. L. Miller, *Origins Life*, 1984, **15**, 7.

[48] A. A. Zharov, G. A. Adadurov, A. G. Kazakevich, V. M. Zulin, and I. I. Zhukuvleva, *Izv. Akad. Nauk S.S.S.R., Ser. Khim.*, 1984, 1199.

[49] Ch. Ivano, O. Ivanov, R. Simeonova, and G. Mirkova, *Origins Life*, 1983, **13**, 97.

Synthesis of Protein Amino Acids and Other Naturally Occurring Amino Acids. – It would be inappropriate to ignore the burgeoning literature, but necessary to give representative citations, only, of fermentative production of amino acids. Enzymic synthesis of amino acids from β-chloroalanine has been reviewed;[50] biosynthetic studies include formation of L-isoleucine by methanogenic bacteria,[51] enhanced L-proline production from L-glutamic acid by a barley mutant,[52] and conversion of L-aspartic acid into L-alanine.[53] The production of L-dopa (*Mucuna pruriens*)[54] and L-tryptophan[55] has been given detailed attention.

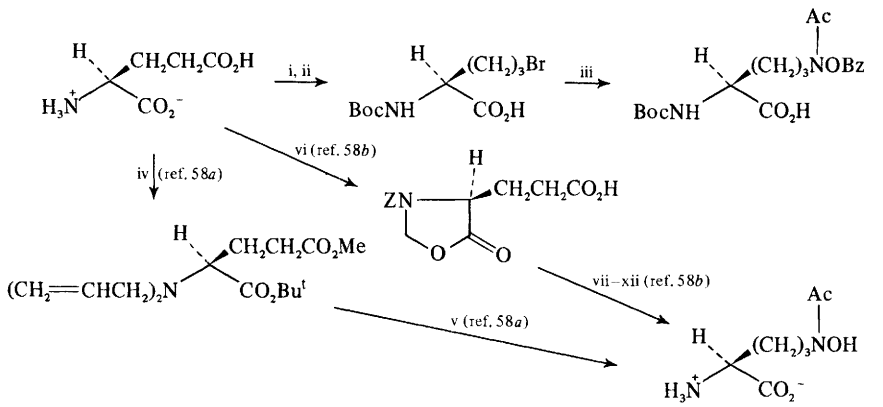
Protein amino acids are frequently the objective of exploratory studies with new or modified general syntheses, and examples of this type appear elsewhere in this chapter. The protein amino acids themselves are starting points for the synthesis of other natural products (see Section 6) including amino acids. The starting protein L-amino acid may appear as such in the synthetic target, as in the synthesis of histopine (5) *via* reductive alkylation of L-histidine with pyruvic acid in the presence of $NaBH_3CN$ and separation of the resulting mixture of diastereoisomers.[56] An alternative synthesis based on the established route to this general class of crown-gall tumour metabolites, in this case using L-histidine and (*R*)- or (*S*)-α-bromopropionic acid, was also explored in this study.[56]



(5)

L-Glutamic acid was the starting point for differently conceived syntheses of N^{δ}-hydroxy-L-ornithine,[57,58] both sketched in Scheme 2. In one of these studies[57] alternative approaches were thwarted by the propensity of the urethane nitrogen atom in N^{α}-Boc-L-glutamic semialdehyde to undergo intramolecular reaction and also by transamidation rearrangements that occurred on attempted reduction of the side-chain carboxy group in certain glutamic acid α-hydroxamate derivatives. However, when the nitrogen atom is enclosed in an oxazolidone ring, this problem is avoided.[58] L-Serine has been used for the synthesis of L-2,3-diaminopimelic acid through application of the Mitsunobo reaction (Z-Ser-OMe with Ph_3P and diethyl or di-isopropyl azodicarboxylate to give the corresponding α-azido-alanine, subjected to H_2S–py reduction).[59] The

[50] T. Nagasawa and H. Yamada, *Kagaku, Zokan (Tokyo)*, 1984, 107.
[51] I. Ekiel, I. C. P. Smith, and G. D. Sprott, *Biochemistry*, 1984, **23**, 1683.
[52] J. S. H. Kueh, J. M. Hill, S. J. Smith, and S. W. J. Bright, *Phytochemistry*, 1984, **23**, 2207.
[53] M. C. Fusee and J. E. Weber, *Appl. Environ. Microbiol.*, 1984, **48**, 694.
[54] H. J. Huizing and H. J. Wichers, *Prog. Ind. Microbiol.*, 1984, **20**, 217.
[55] S. Takao, A. Yokota, and M. Tanida, *J. Ferment. Technol.*, 1984, **62**, 329.
[56] H. A. Bates, A. Kaushal, P. N. Deng, and D. Sciaky, *Biochemistry*, 1984, **23**, 3287.
[57] R. K. Olsen, K. Ramasamy, and T. Emery, *J. Org. Chem.*, 1984, **49**, 3527.
[58] (*a*) G. Benz, *Liebigs Ann. Chem.*, 1984, 1424; (*b*) B. H. Lee and M. J. Miller, *Tetrahedron Lett.*, 1984, **25**, 927.
[59] B. T. Golding and C. Howes, *J. Chem. Res. (S)*, 1984, 1.



Reagents: $BocN_3$; ii, established route; iii, *O*-benzyl acetohydroxamate, NaH; iv, esterify, $CH_2{=}CHCH_2Br$; v, $(Ph_3P)_3RhCl$, CF_3CO_2H, hydrogenolysis; vi, ZCl, CH_2O/TsOH; vii, $SOCl_2$; viii, Bu_3SnH or $Li(OBu^t)_3AlH$; ix, $PhCH_2ONH_2$; x, $NaBH_3CN$; xi, OH^-; xii, HBr

Scheme 2

conversion of L-serine into D-α-amino acids[60] involves *N*-benzenesulphonyl-L-serine lithium salt for aminoacylation of a Grignard reagent, the resulting side-chain carbonyl group being converted into a methylene group through Raney nickel reduction of the derived dithioketal; oxidation ($-CH_2OH \rightarrow -CO_2H$) was achieved using O_2/PtO_2, leading to excellent yields of D-amino acids $H_3\overset{+}{N}CH(CH_2R)CO_2HBr^-$ after cleavage of the N-protecting group with 48% HBr.[60]

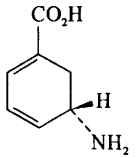
Another example in which a chiral natural product, this time (*R*,*R*)-tartaric acid, serves as starting material for a natural amino acid is the 25-stage synthesis of (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methylamino-6-octenoic acid (a constituent of cyclosporin A).[61]

trans-α-(Carboxycyclopropyl)glycine, a constituent of ackee seed, has been prepared through cyclopropanation of (E)-$EtO_2CCH{=}CHCH(OEt)_2$, conventional amino acid synthesis through the masked aldehyde group involving the Strecker route.[62] Stammer's group (see Vol. 15, p. 12) continue their studies on synthesis of cyclopropane-based amino acids by diazoalkane cyclopropanation of amino-acrylates with examples including coronamic acid.[63]

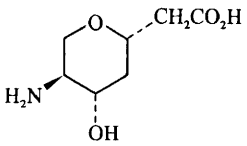
Synthesis of β- and Higher Homologous Amino Acids. – The large range of examples, many of them represented in natural products, that are covered by the title of this section is matched by a constant stream of papers. There are relatively few general methods specific to each class of ω-amino acid, and text-book methods of synthesis of amines are used, needing little particular discussion.

[60] P. J. Mauer, H. Takahata, and H. Rapoport, *J. Am. Chem. Soc.*, 1984, **106**, 1095.
[61] R. M. Wenger, *Helv. Chim. Acta*, 1983, **66**, 2308.
[62] S. R. Landor, P. D. Landor, and M. Kalli, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2921.
[63] J. M. Bland, C. H. Stammer, and K. I. Varughese, *J. Org. Chem.*, 1984, **49**, 1634; M. Suzuki, E. E. Gooch, and C. H. Stammer, *Tetrahedron Lett.*, 1983, **24**, 3839.

3-Ketoglutaric acid is converted through some conventional steps but including a notable use of *Arthrobacter* for stereoselective formation of ethyl (*S*)-3-hydroxyglutarate, into either L-carnitine, $[Me_3\overset{+}{N}CH_2CH(OH)CH_2CO_2^-]$, or the 4-amino-3-hydroxybutanoic acid itself.[64] Other γ-amino acids reached through enantiospecific synthesis are (3*S*,4*S*)-statine,[65] as its *N*-Boc ester, $Me_2CHCH_2CH(NHBoc)CH(OH)CH_2CO_2Me$, starting with *N*-Boc-L-leucinal, and (−)-gabaculine (6), starting with benzoic acid and including a notable role for the $Fe(CO)_3$ moiety in enabling enantiospecific introduction of ^2H as well as the correct location of the amino group.[66] Detoxinine has been synthesized from (S)-$CH_2{=}CHCH_2CH(\overset{+}{N}H_3)CO_2^-$ through a stereoselective route[67] that competes with an alternative route described in Vol. 16, p. 10. The synthesis of (+)-galantinic acid (7) starting with (R)-$CH_2{=}CHCH(\overset{+}{N}H_3)CO_2^-$ mimics the detoxinine synthesis in some respects, involving stereospecific epoxidation and regiospecific ring cleavage with $Li_2(CN)Cu(CH{=}CHCH_2OSiMe_2CMe_3)_2$.[67,68] L-Glutamic acid serves as starting material for (*S*)-4-amino-4,5-dihydrofuran-2-carboxylic acid, found to be a potent γ-aminobutyric acid transaminase inhibitor;[69] so also is (*S*)-4-amino-5-hexenoic acid, prepared from (*S*)-5-vinyl-2-pyrrollidone, available from L-glutamic acid through straightforward elaboration.[70]



(6)



(7)

Synthesis of α-Alkyl Analogues of Protein Amino Acids. – Alkylation reactions continue to gain favour for this purpose as reaction procedures become optimized, in comparison with total synthesis by standard general methods employing ketones. In addition to examples described elsewhere in this chapter,[30,75] alkylation of Schiff bases $R^1R^2C{=}NCHR^3CO_2R^4$ is easily accomplished using an alkyl halide in refluxing MeCN in the presence of K_2CO_3 and $Bu_4N^+Br^-$.[71] α-Alkylated L-leucines are accessible through the use of lithiated 2,5-dimethoxy-3,6-diisobutyl-3,6-dihydropyrazine (see also refs. 44 and 45) as chiral substrate;

[64] A. S. Gopalan and C. J. Sih, *Tetrahedron Lett.*, 1984, **25**, 5235.
[65] B. Rague, J. A. Fehrentz, R. Guegan, Y. Chapleur, and B. Castro, *Bull. Soc. Chim. Fr.*, 1983, 230.
[66] B. M. R. Bandara, A. J. Birch, and L. F. Kelly, *J. Org. Chem.*, 1984, **49**, 2496.
[67] Y. Ohfune, N. Kurokawa, H. Nishio, and M. Matsunaga, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, 1983, 500 (*Chem. Abstr.*, 1984, **100**, 210 365).
[68] Y. Ohfune and N. Kurokawa, *Tetrahedron Lett.*, 1984, **25**, 1587.
[69] J. P. Burkhart, G. W. Hobert, and B. W. Metcalf, *Tetrahedron Lett*, 1984, **25**, 5267.
[70] W. Frieben and F. Gerhart, Brit. U.K. Pat. Appl. GB 2,133,002 (*Chem. Abstr.*, 1984, **101**, 231 027).
[71] M. J. O'Donnell, K. Wojciechowski, L. Ghosez, M. Navarro, F. Sainte, and J. P. Antoine, *Synthesis*, 1984, 313.

alkylation is followed by hydrolysis to give the α-alkyl-leucine methyl ester.[72] Similar use of the chiral oxazinone formed between DL-2-(2-furyl)glycine and (*S*)-Pr^{i}CH(OAc)COCl or (*S*)-Bu^{t}CH(OAc)COCl after conversion into its potassium salt leads to (*S*)-α-alkyl-α-(2-furyl)glycines with asymmetric induction levels of 50–95%.[73]

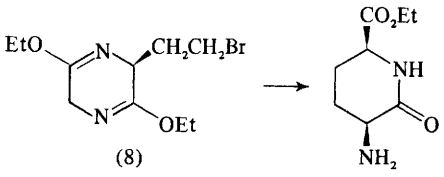
Synthesis of Other Aliphatic, Alicyclic, and Saturated Heterocyclic α-Amino Acids. – The use of 1,2-diaminoethane in the Strecker synthesis yields 'bis-α-amino acids' *via* the amino nitrile:[74]

$$EtCHO + KCN + H_2NCH_2CH_2NH_2 \rightarrow NCCHEtNHCH_2CH_2NHCHEtCN$$

Hydrolysis to the amino acid succeeded only after benzoylation of both secondary amino groups. No such problem arose in the hydrolysis of the nitrile groups in alkylation products of the Schiff base $Ph_2C{=}NCH_2CN$ in a study of bis-alkylation by 1,ω-dibromoalkanes $Br(CH_2)_nBr$ leading to 1-aminocyclopropane-1-carboxylic acid and 'cycloleucine' ($n = 4$) and to 2,6-diaminopimelic acid ($n = 3$).[75]

The acetamidomalonate synthesis in its half-nitrile version has been used in the synthesis of tetaine analogues, using 3-bromomethylenecyclohexene as alkylating agent.[76] β-(2,3-Epoxycyclohexyl)alanine was formed from the hydrolysis-decarboxylation product by standard methods.

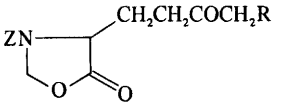
LL-3-Amino-2-piperidine-6-carboxylate (9) has been obtained in better than 99.5% chiral efficiency in an intramolecular alkylation of 2-bromoethyl-3,6-dihydropyrazine (8) followed by acid hydrolysis.[77] This astonishing selectivity may be restricted to this particular example, since higher ω-bromoalkyl homologues would presumably react with competing inter- and intra-molecular alkylation pathways.



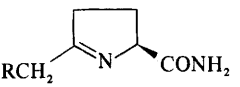
(8) (9)

Ring opening of lactones (10) derived from 2-amino-5-oxoalkanoic acids by treatment with NH_3 is followed by closure to give pyrrolines (11) after removal of the N-protecting group; further steps lead to *cis*-5-alkylprolines in high optical

[72] U. Schöllkopf, U. Busse, R. Kilger, and P. Lehr, *Synthesis*, 1984, 271.
[73] U. Schöllkopf and R. Scheuer, *Liebigs Ann. Chem.*, 1984, 939.
[74] R. Iyer, S. S. Lawate, and M. S. Sonaseth, *Indian J. Chem., Sect. B*, 1984, **23**, 3 (*Chem. Abstr.*, 1984, **101**, 192 413).
[75] M. J. O'Donnell, W. A. Brude, T. M. Eckrich, D. F. Shullenberger, and G. S. Staten, *Synthesis*, 1984, 127.
[76] M. Smulkoski, M. Dzieduszycka, and E. Borowski, *Pol. J. Chem.*, 1982, **56**, 699 (*Chem. Abstr.*, 1984, **100**, 86 098).
[77] D. S. Kemp and P. E. McNamara, *J. Org. Chem.*, 1984, **49**, 2286.



(10)



(11)

yield.[78] The use of one amino acid to synthesize another is also the basis of a route to long-chain α-amino acids starting from L-$BrCH_2CH_2CH(NHBoc)CO_2Me$; reaction with an organocuprate R_2CuLi involves no racemization[79] (see also ref. 300).

Synthesis of ω-Alkoxy-α-Amino Acids. – Anodic methoxylation of methyl *N*-formylprolinate in MeOH at a Pt anode gives the corresponding 5-methoxy compound. The group introduced in this way can be substituted by nucleophiles (dimethyl malonate, or 1,3,5-trimethylbenzene in the presence of $AlCl_3$) to give 5-substituted prolines.[80]

Synthesis of Halogenoalkyl Amino Acids. – Introduction of fluorine atoms into simple amino acids continues to offer considerable chemical interest, and the products are important as potential enzyme inhibitors. Replacement of the hydroxy group of serine using SF_4 has hitherto been difficult to perform in satisfactory yields, now[81] seen to be due to competition by a reaction in which serine and SF_4 combine in a 2:1 ratio. High dilution then became the simple cure for the problem. The other papers selected for review here deal with total synthesis by standard methods: Strecker procedure with 3-fluoro-2-hydroxynitriles and amines *via* 2-amino-3-fluoronitriles (inversion of configuration is notable)[82] and reductive amination of 3-fluoropyruvates $p\text{-}RC_6H_4CHFCOCO_2Na$ (to give the *erythro* isomer predominantly).[83]

Synthesis of Aliphatic α-Amino Acids Carrying Side-chain Hydroxy Groups. – The preparation of the simplest sort under this heading, the aldol-type addition reaction of an aldehyde (or reaction with an acyl chloride, then $NaBH_4$ reduction) with a glycine derivative, continues to provide points of interest. Lithium enolates of di-*N*-benzylglycine esters yield *threo*-β-hydroxy-α-amino acid derivatives $(PhCH_2)_2NCH(CHR^1OH)CO_2R^2$ with remarkably high diastereoselectivity.[84]

[78] T. L. Ho, B. Gopalan, and J. J. Nestor in 'Peptides: Structure and Function', Proceedings of the 8th American Peptide Symposium, ed. V. J. Hruby and D. H. Rich, Pierce Chemical Company, Rockford, Illinois, U.S.A., 1983, p. 147.

[79] J. A. Bajgrowicz, A. El Hallaoui, R. Jacquier, C. Pigiere, and P. Viallefont, *Tetrahedron Lett.*, 1984, **25**, 2231.

[80] M. Malmberg and K. Nyberg, *Acta Chem. Scand., Ser. B*, 1984, **38**, 85.

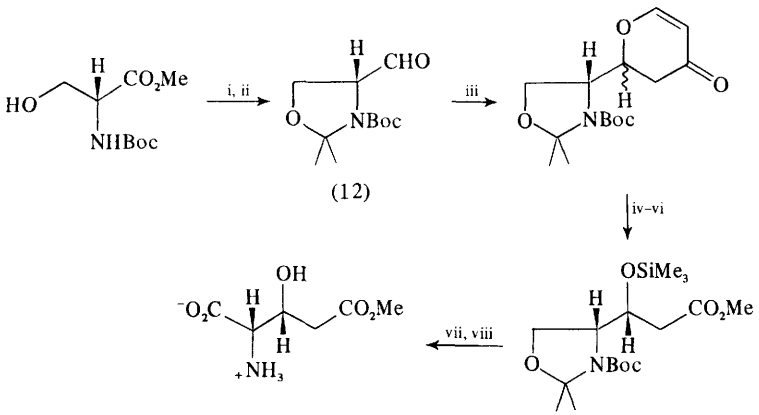
[81] A. W. Douglas and P. J. Reider, *Tetrahedron Lett.*, 1984, **25**, 2851.

[82] A. I. Ayi and R. Guedj, *J. Fluorine Chem.*, 1984, **24**, 137.

[83] T. Tsushima, K. Kawada, J. Nishikawa, T. Sato, K. Tori, T. Tsuji, and S. Misaki, *J. Org. Chem.*, 1984, **49**, 1163.

[84] G. Guanti, L. Banfi, E. Narisano, and C. Scolastico, *Tetrahedron Lett.*, 1984, **25**, 4693.

Hydroxyglutamic acid derivatives have been prepared from glutamic acid itself (*threo*-4-hydroxy-D-glutamic acid, from *N*-phthaloyl 4-bromo-D-glutamic acid dimethyl ester, showed high glutamine synthetase-inhibiting activity[85]) and from L-serine (*threo*-β-hydroxy-L-glutamic acid) starting with a stereoselective addition of the serinal derivative (12) to 1-methoxy-3-(trimethylsilyloxy)-1,3-butadiene (Scheme 3).[86]



Reagents: i, DMP, TsOH; ii, di-isobutylaluminium hydride, −78 °C; iii, MeOCH=CHC(OSiMe$_3$)=CH$_2$; iv, NaIO$_4$–RuO$_2$; v, Et$_2$NSiMe$_3$; vi, MeOH, H$^+$; vii, KMnO$_4$; viii, H$_3$O$^+$Cl$^-$

Scheme 3

Synthesis of Aliphatic α-Amino Acids with Unsaturated Side Chains. – This section covers the logical progression from 'dehydroamino acids' (alias 2-amino-2-alkenoic acids) to vinyl and allenic amino acids, and analogues of well known α-amino acids in which an unsaturated grouping has been introduced.

Serine Schiff bases ArCH=NCH(CH$_2$OH)CO$_2$Me yield imidazolylcarbonyl derivatives with carbonyldi-imidazole, which suffer elimination in the presence of Et$_3$N to give the corresponding dehydroalanine Schiff bases.[87] Oxidative β-decarboxylation of *N*-acyl aspartate α-esters by sodium hypochlorite gives the corresponding *N*-acyl dehydroalanines through *N*-chlorination followed by dehydrochlorination.[88] α-Azidocarboxylic esters treated with Ac$_2$O–Re$_2$S$_7$ give *N*-mono- or -di-acetyl dehydroamino acids,[89] while Ac$_2$O–NaReO$_4$ at 90 °C was appropriate for the similar conversion of α-azidocarboxylic acid amides.[90] Direct introduction of an alkylidene group into a glycine derivative is a way of

[85] V. P. Krasnov, L. V. Alekseeva, N. A. Firsova, I. K. Kodess, and N. L. Burde, *Khim.-Farm. Zh.*, 1984, **18**, 655.
[86] P. Garner, *Tetrahedron Lett.*, 1984, **25**, 5855.
[87] G. Wulff and H. Boehnke, *Angew. Chem.*, 1984, **96**, 362.
[88] M. Seki, T. Moriya, and K. Matsumoto, *Agric. Biol. Chem.*, 1984, **48**, 1251.
[89] F. Effenberger and T. Beisswenger, *Chem. Ber.*, 1984, **117**, 1497.
[90] T. Beisswenger and F. Effenberger, *Chem. Ber.*, 1984, **117**, 1513.

describing the well known azlactone synthesis of amino acids, referred to in an earlier section of this chapter; a novel variant of the approach from a substituted glycine with an aldehyde has been described, employing $(R^3O)_2P(O)CH(NHR^1)$-COR^2, a series of *N*-acyl-2-(dialkyloxyphosphinyl)glycine esters and amides.[91] Condensation of carbonyl compounds with ethyl isocyanoacetate gives 'dehydro-isocyano acids'.[92]

Vinylglycine continues to challenge the application of synthetic methodology and has become available in racemic form from (*Z*)-$HOCH_2CH{=}CHCH_2OH$ through imidation with CCl_3CN followed by [2,3]-sigmatropic rearrangement in refluxing t-butylbenzene then oxidation ($CH_2OH \rightarrow CO_2H$) and hydrolysis[93] (see also ref. 33). The L-enantiomer has been synthesized from *N*-benzyloxycarbonyl-L-glutamic acid α-methyl ester through $Cu(OAc)_2$-catalysed decarboxylative elimination by lead tetra-acetate, followed by deprotection.[94] β-Methyleneaspartic acid has been obtained from 1,1,2-tricarbethoxyprop-1-ene (from diethyl malonate and ethyl pyruvate) through amination with chloramine and ester hydrolysis accompanied by decarboxylation.[95]

α-Allenic amino acids are potent inhibitors of bacterial tyrosine decarboxylase,[96] mammalian 4-aminobutyrate-2-oxoglutarate aminotransferase, and bacterial ornithine decarboxylase.[97] As such, this class of protein amino acid analogue is certain to be the subject of more attention, and serviceable syntheses have been established, either through Claisen rearrangement of α-benzamidopropargylic esters into 2-phenyl-4-allenic oxazolinones followed by methanolysis and hydrolysis[96,97] or from α-acetylenic amino acids (see Vol. 16, p. 14) with formaldehyde in the presence of CuBr and di-isopropylamine.[97]

Synthesis of Aromatic and Heteroaromatic Amino Acids. – The usual theme for this section, synthesis of close analogues of a number of natural aromatic and heteroaromatic α-amino acids, applies again this year.

Standard approaches have been successful for the synthesis of β-(4-aminophenyl)alanine[98] (azlactone synthesis *via* *p*-nitrobenzylidene-oxazolinones), methyldopa[99] (veratraldehyde from vanillin, thence to 3,4-dimethoxyphenylacetone *via* Darzens condensation), and arsanilazo and sulphanilazo derivatives of tyrosine and histidine[100] (electrophilic substitution of the *N*-acetyl amino acids by diazotized arsanilic and sulphanilic acids). *p*-Boronophenylalanine, $4\text{-}(HO)_2BC_6H_4CH_2CH(\overset{+}{N}H_3)CO_2^-$, is accessible through a similar general approach.[101]

[91] U. Schmidt, A. Lieberknecht, and J. Wild, *Synthesis*, 1984, 53.
[92] C. Herdeis and A. Dimmerling, *Arch. Pharm. (Weinheim, Ger.)*, 1984, **317**, 86.
[93] D. M. Vyas, Y. Chiang, and T. W. Doyle, *J. Org. Chem.*, 1984, **49**, 2037.
[94] S. Hanessian and S. P. Sahoo, *Tetrahedron Lett.*, 1984, **25**, 1425.
[95] E. S. Hand and D. C. Baker, *Int. J. Pept. Protein Res.*, 1984, **23**, 420.
[96] A. L. Castelhano, D. H. Pliura, G. J. Taylor, K. C. Hsieh, and A. Krantz, *J. Am. Chem. Soc.*, 1984, **106**, 2734.
[97] P. Casara, K. Jund, and P. Bey, *Tetrahedron Lett.*, 1984, **25**, 1891.
[98] Y. Yuan and C. Yang, *Dalian Gongxueynan Xuebao*, 1984, **23**, 135.
[99] R. Du, X. Luo, and B. Guo, *Jinan Liyi Xuebao*, 1984, **52** (*Chem. Abstr.*, 1984, **101**, 152 292).
[100] G. J. Pielak, M. S. Urdea, K. Igi, and J. I. Legg, *Biochemistry*, 1984, **23**, 589.
[101] T. Hamada, K. Aoki, T. Kobayashi, and K. Kanda, *Annu. Rep. Res. React. Inst., Kyoto Univ.*, 1983, **16**, 112.

Hydriodic acid degradation of the red-brown polymeric pigment phaeomelanin gives (4′-amino-3′-hydroxyphenyl)alanine and (7′-hydroxybenzothiazol-4′-yl)-alanine, amongst other products, and straightforward applications of standard routes to amino acids have been used for their synthesis.[102]

As well as examples in the preceding paragraphs, the heteroaromatic area is represented by the synthesis of substituted tryptophans. 2-(Alkanethio)tryptophans are best obtained[103] *via* 2,3-dihydropyrrolo[2,3-*b*]indoles, easily prepared from L-tryptophan. Electrochemical methoxylation of *N*-methoxycarbonyl-L-proline (see Vol. 16, p. 13) and conventional indolization of the derived pyrrolines into 5-substituted L-tryptophans (after removal of protecting groups, notably removal of the methoxycarbonyl group by Me_3SiI in refluxing chloroform) have been described.[104] 1-Aryl-2-cyanoaziridines are effective electrophiles for reaction at the 3-position of indoles in the presence of BF_3–Et_2O, giving satisfactory yields of nitriles from which *N*-aryltryptophans were obtained.[105]

Synthesis of *N*-Substituted Amino Acids. – This section has a narrower coverage than the title might imply, since protected amino acids for analytical or synthetic use are excluded (some coverage of these appears in the 'Reactions' and 'Analytical Methods' sections later in this chapter).

Alkylation of the Schiff bases $4\text{-}R^3C_6H_4CH{=}NCR^1R^2CO_2Me$ or the amidines $Me_2NCH{=}NCR^1R^2CO_2Me$ by Me_2SO_4, Et_2SO_4, or methyl triflate occurs without racemization.[106] More conventional alkylation procedures have been used for the preparation of 'amino diacids' ($R^5O_2CCR^1R^2NH_2 + BrCR^3R^4CO_2Et \rightarrow R^5O_2CCR^1R^2NHCR^3R^4CO_2Et$)[107] and similar compounds (see earlier sections). The racemization-free route from *N*-benzyloxycarbonylamino acids to *N*-methylamino acids (hydrogenation of lactones formed with formaldehyde) has been applied for the synthesis of *N*-methyl-L-phenylalanine.[108]

The vaguely named *N*-methyl analogue of *S*-adenosylmethionine[109] (*i.e.* MeN in place of S^+Me) has been synthesized from 2′,3′-*O*-(1-methylethylidene)adenosine 5′-tosylate, $MeNH_2$, and $PhCH_2O_2CCH(N_3)CH_2CH_2I$, the product being elaborated by standard methods.

Synthesis of Amino Acids Containing Sulphur or Selenium. – Oxidation of penicillamine with H_2O_2 yields D-ββ-dimethylcysteic acid.[110] The other non-routine examples of syntheses of sulphur-containing amino acids also start with

[102] D. G. Patil and M. R. Chedekel, *J. Org. Chem.*, 1984, **49**, 997.
[103] M. Ohno, S. Tanaka, T. C. Shieh, and T. F. Spande, *J. Org. Chem.*, 1984, **49**, 5069.
[104] K. Irie, A. Ishida, T. Nakamura, and T. Oh-Ishi, *Chem. Pharm. Bull.*, 1984, **32**, 2126.
[105] S. Apparao, G. Singh, H. Ila, and H. Junjappa, *Indian J. Chem., Sect. B*, 1984, **23**, 15 (*Chem. Abstr.*, 1984, **101**, 171 670).
[106] M. J. O'Donnell, W. A. Bruder, B. W. Daugherty, D. Liu, and K. Wojciechowski, *Tetrahedron Lett.*, 1984, **25**, 3651.
[107] B. Garrigues, *Tetrahedron*, 1984, **40**, 1151.
[108] G. Cipens, V. A. Slavinskaya, D. Sile, V. D. Grigorova, D. Kneile, and D. Eglite, *Latv. P.S.R. Zinat. Akad. Vestis, Kim. Ser.*, 1984, 620 (*Chem. Abstr.*, 1984, **102**, 79 293).
[109] M. Davis, N. P. B. Dudman, and H. F. White, *Aust. J. Chem.*, 1983, **36**, 1623.
[110] A. Calvo, R. Faggiani, D. A. Harvey, H. E. Howard-Lock, W. F. Kean, and C. J. L. Lock, *J. Crystallogr. Spectrosc. Res.*, 1984, **14**, 59.

homocysteine sodium salt and a 5′-chloro-5′-deoxynucleoside (syntheses of *S*-adenosyl-L-homocystine and related compounds, including a first synthesis of N^6,N^6-dimethyladenosyl-L-homocysteine[111]) or with the deoxynucleoside itself, condensation to various *S*-nucleosidylhomocysteines being catalysed by *S*-adenosylhomocysteine hydrolase in intact *Alcaligenes faecalis* cells.[112]

Preparations of *m*- and *p*-methylselenylphenylalanines have been reported,[113] the starting point being the corresponding cyanoselenylbenzyl acetamidomalonates (reaction with MeMgBr and hydrolysis in refluxing 12M HCl).

Amino Acids Synthesized for the First Time. – This section is to be taken with the chapter as a whole by readers seeking coverage of newly synthesized amino acids. Amino acids of this description are merely listed here: DL-2-amino-4-(*o*-chlorophenoxy)butanoic acid,[114] N^6-monoethyl-L-arginine,[115] N^{α}-alkyl-N^{β}-phenylcarbamyl-DL-asparagines,[116] (4-bis-chloroethylaminophenyl)alanine,[26] β-(3-pyridyl)-L-alanine,[151] β-ruthenocenylalanine,[117] *m*-cyclohexyloxy-L-tyrosine,[118] (*E*)-3′-hydroxyiminomethyl-L-tyrosine,[119] *S*-[ω-(*o*-alkoxyphenyl)alkyl]homocysteines,[120] and *N*,*N*,*S*-tris(carboxymethyl)-DL-methionine.[203]

Synthesis of Labelled Amino Acids. – This section is organized around the isotopic replacements described, in order of increasing atomic mass. α-Amino acids are covered first.

Deuterium-substituted analogues of protein amino acids can be prepared with secure knowledge of the absolute configuration by straightforward methods; (2*R*,3*R*)-phenylalanine-2,3-$^2H_2$ is available through addition of $^2H_2$ in the presence of Pd to the diketopiperazine of D-alanyldehydrophenylalanine, with better than 98.8% chiral purity.[121] Efficient and specific α-deuteriation of *N*-acetyl-DL-acetylamino acids occurs through equilibration in basic ^2H_2O–Ac_2O at 40 °C; resolution using porcine kidney acylase I gave L-[α-^{2}H]phenylalanine,[122] a compound that could also be prepared using pyridoxal derivatives in basic ^2H_2O.[122]

No problems of selectivity were involved in the synthesis of (*S*)-N^{τ}-methyl-$^2H_3$-L-histidine by methylation with C^2H_3I of the histidine protected at its

[111] K. Ramalingam and R. W. Woodard, *J. Org. Chem.*, 1984, **49**, 1291.
[112] S. Shimizu, S. Shiozaki, T. Oshiro, and H. Yamada, *Agric. Biol. Chem.*, 1984, **48**, 1383.
[113] C. A. Loeschorn, C. J. Kelley, R. N. Hanson, and M. A. Davis, *Tetrahedron Lett.*, 1984, **25**, 3387.
[114] U. Petzold, S. Neumann, F. Jacob, and M. Strube, *Wiss. Beitr. Martin-Luther Univ., Halle-Wittenberg*, 1984, 47 (*Chem. Abstr.*, 1984, **101**, 186 015).
[115] Y. B. Cho, G. Furst, and W. K. Paik, *Anal. Biochem.*, 1984, **139**, 377.
[116] G. A. Zeinalova, N. S. Kyazimova, and E. A. Nagieva, *Dokl. Akad. Nauk Az.S.S.R.*, 1983, **39**, 57 (*Chem. Abstr.*, 1984, **101**, 131 050).
[117] W. H. Soine, C. E. Guyer, and F. F. Knapp, *J. Med. Chem.*, 1984, **27**, 803.
[118] E. Giralt, D. Andreu, R. Eritja, A. Grandas, and E. Pedroso, *An. Quim., Ser. C*, 1983, **79**, 390.
[119] Z. Arnold, *Pol. J. Chem.*, 1983, **56**, 1021.
[120] O. W. Lever, C. Hyman, and H. L. White, *J. Pharm. Sci.*, 1984, **73**, 1241.
[121] K. Tanimura, T. Kato, M. Waki, S. Lee, Y. Kodera, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2193.
[122] H. Fujihara and R. L. Schowen, *J. Org. Chem.*, 1984, **49**, 2819.

N^{π} nitrogen atom by cyclocondensation.[123] Equilibration of phenylalanine and its *p*-OH, -OMe, -OEt, and $-NO_2$ analogues in ^2H_2O–K_2PtCl_4 brought about exchange of aromatic and CH_3 protons preferentially.[124]

^2H- and ^{13}C-labelled prolines have been described,[125] the [4,4-$^2H_2$]imino acid from ethyl acetamidocyanoacetate and $BrCH_2C^2H_2CH_2Br$ and the [4-^{13}C] analogue in a corresponding manner.

^{11}C-Labelled amino acids continue to excite interest, not only because of their value in imaging studies but also in the need for rapid syntheses, bearing in mind the relatively short half-life of this isotope. A total synthesis time of 46 min is reported for [1-^{11}C]dopa prepared by carboxylation of 3,4-$(MeO)_2$-$C_6H_3CH_2CHLiNC$, including 16 min for h.p.l.c. resolution.[126] [3-^{11}C]Phenylalanine and [3-^{11}C]dopa in racemic forms have been prepared through the azlactone route,[127] and an alternative route to [3-^{11}C]phenylalanine[128] uses $Ph^{11}CH_2Cl$ for the alkylation of $Ph_2C{=}NCH_2CO_2Et$. Similar alkylations [of $Me_2NCH{=}NCH(Pr^i)CO_2Me$] with $^{11}CH_3I$ gave DL-α-[^{11}C]methyl valine[129] and DL-α-[^{11}C]methyl ornithine [by methylation of the N^{α}-(*p*-nitrobenzylidene)-ornithine lactam].[129]

Strecker syntheses of aspartic acid-1-^{13}C and glutamic acid-1-^{13}C (EtO_2CCH_2-CHO and $EtO_2CCH_2CH_2CHO$, respectively, with $K^{13}CN/NH_4OH/NH_4Cl$) proceed in 45% and 85% yields, respectively.[130] Glutamic acid-3-^{13}C was prepared from $PrO_2CCH_2{}^{13}CH_2Br$ through the acetamidomalonate route.[130]

DL-[2-^{13}C,^{15}N](*p*-Hydroxyphenyl)glycine, for use in nocardicin A biosynthesis studies, has been synthesized through Vilsmeier reaction of anisole with [^{13}C]-dimethylformamide and then the use of the resulting benzaldehyde with $^{15}NH_4Cl$, NaCN, and MeOH at 40 °C in the Strecker procedure.[131] DL-Glutamine-2,5-$^{15}N_2$ has been prepared from α-ketoglutaric acid through reaction with $^{15}N_2H_4$ and hydrogenation of the resulting cyclic hydrazone in the presence of Pd. The synthesis of ^{15}N-amino acids has been reviewed briefly.[133]

6-[^{18}F]Fluoro-L-dopa is available, though in low yields, through reaction of $AcO^{18}F$ with 3-methoxy-4-hydroxy-L-phenylalanine ethyl ester hydrochloride followed by removal of protecting groups.[134]

[123] S. S. Yuan and A. M. Ajami, *J. Labelled Compd. Radiopharm.*, 1984, **21**, 97.
[124] M. Kanska, *J. Radioanal. Nucl. Chem.*, 1984, **87**, 95.
[125] P. E. Young and D. A. Torchia in 'Peptides: Structure and Function', Proceedings of the 8th American Peptide Symposium, ed. V. J. Hruby and D. H. Rich, Pierce Chemical Company, Rockford, Illinois, U.S.A., 1983, p. 155.
[126] J. M. Bolster, W. Vaalburg, W. Van Veen, T. Van Dijk, H. D. Van der Molen, H. Wynberg, and M. G. Woldring, *Int. J. Appl. Radiat. Isot.*, 1983, **34**, 1650.
[127] C. Halldin and B. Laangstroem, *Int. J. Appl. Radiat. Isot.*, 1984, **35**, 779.
[128] M. R. Kilbourn, D. D. Dischino, and M. J. Welch, *Int. J. Appl. Radiat. Isot.*, 1984, **35**, 603.
[129] F. Oberdorfer, *Int. J. Appl. Radiat. Isot.*, 1984, **35**, 559.
[130] U. Fotader and D. Cowburn, *J. Labelled Compd. Radiopharm.*, 1983, **20**, 1003.
[131] C. A. Townsend and G. M. Salituro, *J. Chem. Soc., Chem. Commun.*, 1984, 1631.
[132] W. M. Lagna and P. S. Callery, *J. Labelled Compd. Radiopharm.*, 1984, **21**, 337.
[133] H. Engelmann, A. Dauert, H. Niclas, and H. Lueneberger, *Zfl. Mitt.*, 1983, **77**, 92 (*Chem. Abstr.*, 1984, **101**, 103 826).
[134] R. Chirakal, G. Firnau, J. Couse, and E. S. Garnett, *Int. J. Appl. Radiat. Isot.*, 1984, **35**, 651.

Chirally deuteriated (*R*)-*N*-trifluoroacetyl-β-alanine-β-^{2}H has been prepared from (*S*)-PhCH^{2}HCH$_2$OH *via* RuO_4 oxidation of the derived (*S*)-PhCH^{2}HCH$_2$-NHCOCF$_3$.[135]

Resolution of Amino Acids. – This topic has settled into regularly populated subsections, covering the various categories of the established resolution techniques.

Schiff bases formed between DL-amino acids and (+)-(*R*,*R*,*R*)- or (−)-(*S*,*S*,*S*)-2-hydroxypinan-3-one can be separated cleanly by column chromatography and converted into the amino acids by mild acid hydrolysis.[136] Alternative chiral derivatization procedures have been explored, in particular the use of 1-(4-substituted phenylsulphonyl)-L-prolyl chlorides, in conjunction with liquid-chromatographic separation.[137]

Chromatographic separation of DL-amino acids over a chiral stationary phase offers a well studied variation, and a new study of the use of cellulose chromatography for the purpose adds to a lengthy list of papers describing this method. Resolution of representative amino acids (seventeen examples) is fully described in this report.[138] Man-made rather than natural chiral solids feature in resolutions of *threo*- and *erythro*-β-hydroxyaspartic acids (L-lysine within the stationary phase gives optimal separation of enantiomers for the *threo* diastereoisomer whereas L-ornithine is more appropriate for the *erythro* isomer).[139] Bonding of an L-amino acid (best results with L-pipecolic acid) to silica gel can be accomplished by first derivatizing the silica with 3-glycidoxypropyltrimethoxysilane, as the basis for resolution of DL-amino acids on the ligand-exchange chromatography principle.[140] A very similar approach, the bonding of *N*-formyl-L-isoleucine or -L-valine to aminopropylated silica gel, has also proved effective in liquid-chromatographic resolution.[141] α-(6,7-Dimethyl-1-naphthyl)isobutylamine bonded to a low-polarity stationary phase proved satisfactory for resolution of *N*-3,4-dinitrobenzoyl-DL-amino acids and other bifunctional amines.[142]

Incorporation of a chiral solute into an otherwise conventional liquid-chromatographic system is an established variant of the principle just reviewed, but the first instance of enantioselectivity based on energy differences of intermolecular hydrogen bonds between the chiral additive and the respective enantiomers has now been claimed.[143] In this case, *N*-acetyl-L-valine t-butylamide in $CHCl_3$–n-hexane was an effective chiral mobile phase for the resolution of *N*-acetyl-DL-amino acid t-butyl esters. The complexation principle with a

[135] R. K. Hill, S. R. Prakash, and T. M. Zydowsky, *J. Org. Chem.*, 1984, **49**, 1666.
[136] J. A. Bajgrowicz, B. Cossec, C. Pigiere, R. Jacquier, and P. Viallefont, *Tetrahedron Lett.*, 1984, **25**, 1789.
[137] C. R. Clark and J. M. Barksdale, *Anal. Chem.*, 1984, **56**, 958.
[138] S. Yuasa, M. Itoh, and A. Shimada, *J. Chromatogr. Sci.*, 1984, **22**, 288.
[139] S. Anpeiji, Y. Toritani, K. Kawada, S. Kondo, S. Murai, H. Okai, H. Yoshida, and H. Imai, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2994.
[140] G. Guebitz, F. Juffmann, and W. Jennenz, *Chromatographia*, 1982, **16**, 103.
[141] J. N. Akanya, S. M. Hitchen, and D. R. Taylor, *Chromatographia*, 1982, **16**, 224.
[142] W. H. Pirkle and M. H. Hyun, *J. Org. Chem.*, 1984, **49**, 3043.
[143] A. Dobashi and S. Hara, *J. Chromatogr.*, 1983, **267**, 11.

copper(II) salt of *N*-n-dodecyl-L-proline as a chiral solute in counter-current liquid–liquid chromatography has proved effective for the clean resolution of DL-isoleucine.[144]

Resolution based on differential solubility of systems formed by each enantiomer with a non-bonded chiral entity has taken interesting forms in recent years, extending the standard techniques based on diastereoisomeric salt formation. However, the success or otherwise of these newer methods is still dependent on the physical nature of the systems; thus, methylammonium salts of *N*-acetyl-DL-phenylalanine cannot be resolved by preferential crystallization (seeding with crystals of the desired enantiomer) whereas ethylammonium and 1,1,3,3-tetramethylbutylammonium homologues are shown to form racemic compounds at melting temperatures (but racemic mixtures at room temperature) and therefore can be resolved by this method.[145] Precipitates formed in aqueous L-phenylalanine and DL-valine, DL-leucine, or DL-isoleucine favour the aliphatic D-amino acids, and optical purities of 84–100% have been determined for the D-amino acids recovered from these 1:1 adducts.[146*a*] A similar approach[146*b*] employing the ternary (1:1:1) complex (D-alaninato)(L-isoleucinato)copper(II) led to the resolution of DL-alanine using L-isoleucine and copper(II) acetate in aqueous solution. A further example (the patent literature is not routinely scanned in preparing this chapter) is the resolution of DL-amino acids by diastereoisomeric salt formation with (+)-α-phenylethanesulphonic acid.[147]

The discovery that preferential adsorption of D-amino acids occurs from solutions of DL-amino acids in contact with clay (montmorillonite) has inspired further studies of this type (see Vol. 14, p. 16) with the use of pressure-jump relaxation methods.[148] Arginine that has intercalated the interlamellar layer of montmorillonite undergoes slow hydrolysis into ornithine and urea.[148*a*] Ion-exchange properties of a magnesium hydrotalcite compound were similarly enantioselective, with L-histidine intercalation being favoured at the expense of its D-isomer.[148*b*]

Enantioselective occlusion into centrosymmetric crystals of glycine accounts for the results obtained with aqueous solutions of DL-amino acids,[149] on the basis that the crystals float in such a way that only one face is available for occlusion and that addition of a particular enantiomer of a hydrophobic amino acid orients the floating crystals so that the face that occludes amino acids of opposite chirality is exposed to the solution. This curious involvement of an

[144] T. Takeuchi, R. Horikawa, and T. Tanimura, *J. Chromatogr.*, 1984, **284**, 285.

[145] T. Shiraiwa, H. Miyazaki, A. Ikawa, and H. Kurokawa, *Nippon Kagaku Kaishi*, 1984, 1425; for similar phase-diagram studies with DL-α-phenylglycine sulphate see T. Shiraiwa, A. Ikawa, K. Fujimoto, K. Iwafuji, and H. Kurokawa, *ibid.*, p. 765.

[146] (*a*) T. Shiraiwa, A. Ikawa, K. Sakaguchi, and H. Kurokawa, *Chem. Lett.*, 1984, 113; *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2234; (*b*) T. Shiraiwa, H. Fukuoka, M. Yoshida, and H. Kurokawa, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 1675.

[147] I. Chibata, S. Yamada, C. Hongo, and R. Yoshioka, Eur. Pat. Appl. EP 119,804 (*Chem. Abstr.*, 1984, **101**, 231 031).

[148] (*a*) T. Ikeda and T. Yasunaga, *J. Phys. Chem.*, 1984, **88**, 1253; (*b*) T. Ikeda, H. Amoh, and T. Yasunaga, *J. Am. Chem. Soc.*, 1984, **106**, 5772.

[149] I. Weissbuch, L. Addadi, Z. Berkovitch-Yellin, E. Gati, M. Lahav, and L. Lieserowitz, *Nature (London)*, 1984, **310**, 161.

air–water interface has been suggested[149] to account for the generation and amplification of optical activity in an initially racemic system.

Enzymic resolution of DL-amino acids continues to be the method of choice for many workers, particularly methods that allow the recovery of both enantiomers. The use of subtilisin Carlsberg, for example,[150] for the catalysed hydrolysis of (*R*,*S*)-*N*-acetylphenylalanine methyl ester into the (*S*)-*N*-acetylphenylalanine and unchanged (*R*)-enantiomer, and applied similarly[151] in synthesis of D- and L-β-(3-pyridyl)alanine, illustrates this principle. The hydrolysis of L-leucyl-γ-substituted glutamates by leucine aminopeptidase provides an effective means of resolution based on the same principle, with *threo*-γ-methyl- and *threo*-γ-fluoro-glutamates but not with the *erythro* isomers.[152]

Whole-cell studies include the use of *Streptomyces zaomyceticus* [catalysed hydrolysis of *N*-benzyloxycarbonyl-(*p*-hydroxyphenyl)glycine into the unprotected L-amino acids without affecting the D-enantiomer[153]] and of *Erwinia carotovora* immobilized in κ-carrageenan gel for the catalysed hydrolysis of the L-enantiomer of *N*-acetyl-DL-methioninamide.[154]

Absolute Configuration Studies. – While enzyme stereospecificity, as exploited in resolution studies (preceding section), has yielded absolute configurational assignments, classical chemical correlation studies continue to be used routinely. 4-Hydroxy-4-methylglutamic acids that occur naturally have hitherto been assigned the (2*S*,4*R*) and (2*S*,4*S*) configurations – wrongly, it now appears, through combined degradative and enzymic studies.[155] Whereas the response of the natural amino acids to L-amino acid oxidase confirms the (2*S*) assignment, oxidative deamination and decarboxylation using $Ca(OCl)_2$ give (*R*)-(—)- or (*S*)-(+)-citramalic acids of known absolute configuration. Absolute configuration was assigned to (2*S*,4*S*)-hydroxyglutamic acid through the same approach, the resulting L-malic acid being identified by optical rotation and response to L-malic acid dehydrogenase.[155]

5 Physical and Stereochemical Studies of Amino Acids

Crystal Structures of Amino Acids and Their Derivatives. – Some of the reports[83,156–159] briefly listed here combine *X*-ray crystallographic studies with

[150] J. M. Roper and D. P. Bauer, *Synthesis*, 1983, 1041.
[151] K. Folkers, T. Kubiak, and J. Stepinski, *Int. J. Pept. Protein Res.*, 1984, **24**, 197.
[152] S. Bory, J. Dubois, M. Gaudry, A. Marwuet, L. Lacombe, and S. Weinstein, *J. Chem. Soc., Perkin Trans. 1*, 1984, 475.
[153] Y. Suhara, S. Itoh, K. Yokose, R. Ninomiya, K. Watanabe, and H. B. Maruyama, *Can. J. Microbiol.*, 1984, **30**, 1301.
[154] Y. Nishida, K. Nabe, S. Yamada, and I. Chibita, *Enzyme Microbiol. Technol.*, 1984, **6**, 85.
[155] B. Bjerg, O. Olsen, and H. Soerensen, *Acta Chem. Scand., Ser. B*, 1983, **37**, 321.
[156] I. Buchanan, M. Minelli, M. T. Ashby, T. J. King, J. H. Enemark, and C. D. Garner, *Inorg. Chem.*, 1984, **23**, 495.
[157] D. L. Eng-Wilmot, A. Rahman, J. V. Mendenhall, S. L. Grayson, and D. Van der Helm, *J. Am. Chem. Soc.*, 1984, **106**, 1285.
[158] C. P. Huber, P. R. Carey, S. C. Hsi, H. Lee, and A. C. Storr, *J. Am. Chem. Soc.*, 1984, **106**, 8263.
[159] G. Valle, G. M. Bonora, and C. Toniolo, *Can. J. Chem.*, 1984, **62**, 2661.

other physical methods and are therefore mentioned again in later sections of this chapter. Further attention has been given (see Vol. 15, p. 20) to three-centre (bifurcated) hydrogen bonds in amino acid crystals involving the $\overset{+}{N}H_3$ group, through assessment of more than 50 structures.[160] A relatively high proportion of three-centre bonds is concluded to be due to a deficiency in the number of functional protons needed to satisfy the normal acceptor co-ordination of the carboxylate oxygens (two per oxygen) and the chloride ions (four per Cl).

Amino acid derivatives that have been subjected to crystal-structure determination include the following: *N*-carboxy-L-valine anhydride,[161] N^{α}-acetyl-L-arginine methyl ester hydrochloride,[162] 1-lithio-3,6-diethoxy-2,5-dimethyl-1,2-dihydropiperazine (the functionalized alanine diketopiperazine derivative of the type used in asymmetric synthesis of α-methyl-α-amino acids; *cf.* refs. 42, 44, 45, and 76),[163] *threo*- and *erythro*-3-fluorophenylalanine,[83] the L-cysteine methyl complex MoO_2(L-Cys-OMe)$_2$,[156] ferric neurosporin-6MeCN (containing the amino acid moiety N^{α}-acetyl-N^{δ}-hydroxy-N^{δ}-[(*R*)-3-hydroxybutyryl]-D-ornithine),[157] *N*-benzoylglycine *S*-ethylthioester and its *N*-(β-phenylpropionyl) analogue (evidence of N···S interaction),[158] and Fmoc-L-alanine and -α-amino-isobutyric acid.[159] In the last-mentioned study, a result emerges from the structural analysis that is relevant to a practical observation: that the unexpected removal of the Fmoc-protecting group by catalytic hydrogenolysis might be explained by the long C(sp^3)—O bond determined for the Fmoc moiety.[159]

Nuclear Magnetic Resonance Spectrometry. – N.m.r. studies of common amino acids themselves mainly concern the pushing back of frontiers in terms of n.m.r. rather than advancing structural knowledge of the amino acids. Solid-state ^{13}C n.m.r. with cross-polarization and magic-angle spinning of alanine and its derivatives reveals specific downfield shifts in methyl resonances due to van der Waals' interactions.[164] Other solid-state studies concern phenylalanine (partial collapse of dipolar and chemical-shift tensors for carbon atoms on and off the C_2 symmetry axis confirms that some sites in one of the crystal modifications permit high-frequency 180° ring flips, or, in other words, loosely packed crystals allow molecular reorientations to occur),[165] and ^2H n.m.r. has been applied to deuteriated α-glycine, to suggest a weak C—H···O hydrogen bond.[166]

Solution studies of more familiar types to regular readers of this *Specialist Periodical Report* deal with ^{13}C n.m.r. demonstration of the adoption by *N*-Boc-sarcosine esters of *cis* and *trans* isomers involving the urethane bond and with a

[160] G. A. Jeffrey and J. Mitra, *J. Am. Chem. Soc.*, 1984, **106**, 5546.
[161] H. Kanazawa, Y. Ohashi, and Y. Sasada, *Acta Crystallogr., Sect. C*, 1984, **40**, 1094.
[162] M. Coll, X. Solans, M. Font-Altaba, and J. A. Subirana, *Int. J. Pept. Protein Res.*, 1984, **23**, 242.
[163] D. Seebach, W. Bauer, J. Hansen, T. Laube, W. B. Schweizer, and J. D. Dunitz, *J. Chem. Soc., Chem. Commun.*, 1984, 853.
[164] C. F. Brewer, *Eur. J. Biochem.*, 1984, **143**, 363.
[165] J. Schaefer, E. O. Stejskal, R. A. McKay, and T. Dixon, *J. Magn. Reson.*, 1984, **57**, 85.
[166] C. Mueller, W. Schajor, H. Zimmermann, and U. Haeberlen, *J. Magn. Reson.*, 1984, **56**, 235.

similar behaviour for *N*-glycylsarcosine (no such splitting of the CO carbon resonance is seen in leucine, alanine, and glycine analogues).[167] β-Deuteriated histidine methylamide and *N*-acetylhistidine and its ethyl ester and methylamide of known absolute configuration were used to assign the β-proton resonances for the normal (non-deuteriated) species;[168] lower- and higher-field components of the β-proton resonances are assigned to the pro-*R*- and pro-*S*-protons, respectively, in deuteriated polar solvents and the other way round in non-polar solvents. Rotamer populations about the C^{α}—C^{β} bond depend little on the state of ionization of the basic and acidic groups but greatly on solvent polarity (similar results were found for Phe, Trp, and Tyr derivatives). Stabilizing interactions between a fluorine atom and an NH_2 group are important in determining conformations of β-fluoro-α-amino acids, even overriding other apparently dominating effects, and have been seen in action with *erythro*- and *threo*-3-fluorophenylalanines.[83] A 180° dihedral angle about the C^{α}—C^{β} bond has been established for (3*R*)-^{2}H-D-phenylalanine-α-^{2}H.[121] On the more general question of precise values of vicinal coupling constants (as used to obtain the foregoing conformational information), values of ^{13}C component vicinal coupling constants have been calculated for the three minimum-energy staggered rotamers for the $C^{\alpha}HC^{\beta}H_2$ side chains of amino acids.[169]

Solution studies also lend themselves to some of the more state-of-the-art variants of n.m.r. spectrometric techniques, with longitudinal and transverse ^1H relaxation rates for L-histidine in water,[170] ^2H relaxation studies to reveal intra- and inter-molecular interactions for simple amino acids in aqueous solution,[171] and ^{13}C spin-lattice relaxation times for serine, threonine, phosphoserine, and phosphothreonine, to indicate that phosphorylation causes only minor motional changes for serine and threonine,[172] and for L-aspartic acid, L-alanine, phosphoserine, and 2-mercapto-L-succinic acid in the presence of paramagnetic metal ions (Cu^{2+} and Mn^{2+}), to indicate sites of co-ordination (Asp-Cu^{2+} involves the amino and β-carboxy groups; Asp-Mn^{2+} involves only the two carboxy groups).[173] ^{13}C spin exchange occurs rapidly in natural-abundance samples of L-isoleucine hydrochloride with magic-angle sample spinning; this important observation allows resonances from carbon atoms bonded together to be identified and to have their chemical shifts correlated.[174] It also allows the detection of carbon atoms that are near to each other in space, even when they are on separate residues.[174]

Conformational studies of the γ-amino acid statine and its analogues indicate a dihedral angle near 165° or 0° for the NH—C_4H bond and near 90° for the

[167] J. M. Matsoukas, *Spectrosc. Lett.*, 1984, **17**, 21.
[168] J. Kobayashi, T. Hifashijima, and T. Miyazawa, *Int. J. Pept. Protein Res.*, 1984, **24**, 40.
[169] M. C. Reddy, B. P. N. Reddy, K. R. Sridharan, and J. Ramakrishna, *Org. Magn. Reson.*, 1984, **22**, 464.
[170] C. Rossi, L. Pogliani, F. Laschi, and N. Niccolai, *J. Chem. Soc., Faraday Trans. 1*, 1983, **79**, 2955.
[171] Y. Van Haverbeke, R. N. Muller, and L. Van der Elst, *J. Phys. Chem.*, 1984, **88**, 4978.
[172] L. Pogliani, N. Niccolai, and C. Rossi, *Spectrosc. Lett.*, 1984, **17**, 159.
[173] S. Khazaeli and R. E. Viola, *J. Inorg. Biochem.*, 1984, **22**, 33.
[174] M. H. Frey and S. J. Opella, *J. Am. Chem. Soc.*, 1984, **106**, 4942.

C_4H—C_3H bond.[175] Four possible conformations are compatible with the n.m.r. data for *N*-Boc-statine methyl ester in solution (although molecular-mechanics calculations strongly favour one of these over the others).

N.m.r. studies involving nuclei with higher atomic numbers continue to make progress, ^{14}N and ^{2}H n.q.r. spectra of cytosine complexes of *N*-formylglycine, *N*-benzoylglycine, and *N*-phthaloylglutamic acid giving electric-field gradient parameters to all ^{14}N and ^{2}H atoms in the molecules.[176] Amongst these heavier nuclei, ^{19}F has long held a place for routine n.m.r. work, applied in this context to *p*-fluorobenzoylamino acids.[177] ^{95}Mo n.m.r. contributes to a multi-pronged study of the cysteine complex MoO_2(L-Cys-OMe)$_2$.[156]

Circular Dichroism. — Thorough studies of N^{α}-acetylamino acid methylamides aimed at interpretations of c.d. data in terms of conformation[178] and the influence of solvent polarity indicate complex equilibria for the aliphatic protein amino acids L-alanine and L-leucine. While L-valine shows similar behaviour in many polar solvents, it behaves like the non-protein amino acid t-butylglycine in fluorinated alcohols in its adoption of a right-handed α-helical conformation.

Established configurational relationships for the two geometrical isomers of the *N*-oxide of *N*-benzyl-L-proline can be transferred to the *N*-oxides of *N*-benzyl-*N*-methyl-L-amino acids through the signs of characteristic Cotton effects.[179]

C.d. in conjunction with other physical methods has contributed to the structure determination of ferric neurosporin, a minor siderophore-like compound containing N^{δ}-hydroxy-D-ornithine; the Λ-*cis* absolute configuration about the central ferric ion and the assignment of the D-configuration to the amino acid moiety followed from empirical correlations of Cotton-effect behaviour.[157]

Mass Spectrometry. — All the papers reviewed here deal with the newer instrumental possibilities, from which the analysis of amino acids and peptides in particular has benefited dramatically, especially the soft-ionization techniques compatible with relatively involatile samples. Even so, some of these 'newer methods' have now become routine in the amino acid field.

Fast-atom-bombardment spectra of samples as small as 10^{-7} g isolated from thin-layer chromatograms were unambiguous criteria for identification,[180] and for *N*-Boc-amino acids there are characteristic features, including a McLafferty-type rearrangement.[181] F.a.b. spectra show more fragmentation, compared with field-desorption mass spectra, for protonated arginine, for which the site of

[175] D. H. Rich, Y. Terada, and M. Kawai, *Int. J. Pept. Protein Res.*, 1983, **22**, 325.
[176] E. A. Keiter, Y. Hiyama, and T. L. Brown, *J. Mol. Struct.*, 1983, **111**, 1.
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[179] I. Z. Siemion, K. Marks, and A. Sucharda-Sobczyk, *Bull. Pol. Acad. Sci., Chem.*, 1983, **31**, 1.
[180] G. D. Tantsyrev, M. I. Povolotskaya, and V. A. Saraev, *Bioorg. Khim.*, 1984, **10**, 848.
[181] G. V. Garner, D. B. Gordon, L. W. Tetler, and R. D. Sedgwick, *Org. Mass Spectrom.*, 1983, **18**, 486.

ammonia loss after ionization is the guanidinium grouping.[182] Both positive- and negative-ion modes were adopted in some of these studies[181,182] and in chemical-ionization spectrometry of the aromatic protein amino acids.[183] Fast heavy-ion-induced desorption of valine and leucine represents a study of damage cross-sections of biologically important molecules for its own sake, rather than advocating the virtues of a technique that is impossibly expensive for routine laboratory work (at least as of 1985).[184]

Secondary-ion mass spectra of amino acids embedded in a glycerol solution of camphorsulphonic acid as matrix have been reported.[185]

Creation of a supersonic molecular beam of tryptophan molecules by combined thermospray and seeded molecular-beam techniques, then photo-ionization, employs a number of commercially available mass-spectrometer accessories.[186]

Infrared and Raman Spectrometry. – Routine i.r. spectrometry has been used in establishing crystal types deposited from supersaturated DL-amino acid solutions[145] (other routine uses are excluded from this section). Intermolecular association (between N and S atoms) has been established for *N*-acylglycine ethylthiolesters[158] by Fourier-transform i.r. studies, and similar self-association occurs with peptide oxazolin-5(4*H*)-ones based on α-aminoisobutyric acid[187] and for phospho-L-serine (hydrogen bonds with the carboxylate group as acceptor).[188] Far-i.r. spectra (30–650 cm^{-1}) of the 22 naturally occurring amino acids (*sic*) accumulated by the Fourier-transform technique[189] have been reported, with as yet little progress in interpretation of the data. The same complexity arises with a similar study, this time[190] based on one amino acid derivative, *S*-nitroso-L-cysteine. Data for isotopically substituted ($S^{15}NO$ and N^2H_3) analogues assisted the full assignment of absorption features by use also of the available potential-energy distribution data; of a total of 96 fundamentals occurring above 300 cm^{-1}, 65 were classified as group vibrations by the potential-energy criterion.

An enterprising study[191] reveals the trend from zwitterionic to 'free-amino-free-carboxy' tautomer with rising temperature for *N*-dialkylglycines and glycine itself.

[182] J. J. Zwinselman, N. M. M. Nibbering, J. Van der Greef, and M. C. Ten Noever de Brauw, *Org. Mass Spectrom.*, 1983, **18**, 525.

[183] T. Hayashi, H. Naruse, Y. Iida, and S. Daishima, *Shitsuryo Bunseki*, 1983, **31**, 205 (*Chem. Abstr.*, 1984, **100**, 210 366).

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[191] M. A. Peterson and C. P. Nash, *J. Phys. Chem.*, 1985, **89**, 522.

Tyrosine and iodotyrosine Raman resonance intensity studies reveal exaltation of certain lines on ionization of the phenolic OH group, suggesting that an ionized residue in the presence of un-ionized tyrosines in a peptide might be located.[192]

Other Physical Studies. – Most of the studies surveyed in this section involve purely physico-chemical measurements: partial molar volumes of α-amino acids in water,[193] viscosity studies,[194–196] including indications of amino acid–detergent interactions[194] through volume changes for solutions of β-alanine, histidine, or glutamic acid,[197] volume and adiabatic compressibility of amino acids in water–urea mixtures,[198] hydration numbers determined from ultrasonic velocity and density data,[199] monolayer formation from long-chain *N*-acyl-L-amino acids,[200] dissociation constants,[201–203] differential-scanning calorimetric evidence for solid-state phase transitions,[204,205] including correction of earlier structural assignments deduced from crystal structures,[205] and electron transfer between metal atoms in cobalt(III)–ruthenium(III) complexes, *e.g.* $[(NH_3)_4Ru(SO_4)\text{-py-CO-X-OCo}(NH_3)_5]$, where X is an amino acid residue.[206]

Other physical studies range through electron microscopy [helical aggregates of chiral *N*-(2-hydroxydodecyl)amino acids[207]], e.s.r. studies of amavadin, bis-[*N*-hydroxy-*N*-(1-carboxyethyl)alaninato]oxovanadium(IV),[208] luminescence and excitation luminescence spectra of powdered DL-tryptophan, L-tyrosine, and DL-phenylalanine,[209] stoicheiometric complex formation between β-alanine or γ-aminobutyric acid and DNA, partly to displace phosphate counter-ions and to alter the DNA–water interactions,[210] and a wholly biological study, the transport

[192] M. H. Baron, C. De Loze, T. Mejean, M. J. Coulange, P. Y. Turpin, and L. Chinsky, *J. Chim. Phys. Phys.-Chim. Biol.*, 1983, **80**, 729.
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of imino and non-α-amino acids across the brush-border membrane of guinea-pig small intestine.[211]

Molecular-orbital Calculations. – A number of glycine-based studies have appeared, some dealing with aspects not easily amenable to experimentation. Conformational analysis of glycine aldehyde[212] (potential-energy curve for N—C—C=O torsion is very similar to that calculated for glycine methyl ester[213]) has been reported, also for glycine itself[214] for aqueous solutions, including both zwitterionic and neutral tautomers. Aqueous hydration of the glycine zwitterion at 25 °C has been simulated by Monte Carlo methods,[215] and calculations have indicated two pathways, one concerted, the other two-step, for the reaction of glycine with ammonia in the presence of Mg^{2+} ions.[216]

Calculations along similar lines yield data for serine–water interactions,[217] free energy of transfer of amino acids from water to apolar solvents (leading to the definition of the apolar surface being of common amino acids),[218] hydrogen-bond energies for the acidic and basic protein amino acids,[219] and the aromatic and heteroaromatic protein amino acids[220] with bases and base pairs of nucleic acids and with conformational-energy features for *N*-acetylalanine methylamide.[221]

6 Chemical Studies of Amino Acids

Racemization. – Results of mechanistic interest continue to accumulate under this heading, as well as applications of data for dating fossils.

A salutary example with a message for those preparing samples for careful determination of enantiomer ratios is the observed mechanochemical racemization of L-leucine in a ball-mill in the presence of such diluents as SiO_2 with dilute hydrochloric acid.[222] Salts of optically active amino acids with mineral acids or sulphonic acids underwent racemization on heating in acetic acid at 80–100 °C

[211] G. M. Hanozet, B. Gordana, P. Parenti, and A. Geuvritore, *J. Membr. Biol.*, 1984, **81**, 233; B. G. Munck, *Biochim. Biophys. Acta*, 1984, **770**, 35.
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[222] A. Ikekawa and S. Hayakawa, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 889.

during 1 hour in the presence of 0.1 molar equivalents of an aldehyde.[223] Some success was achieved[223] in an attempted asymmetric transformation involving the preferential crystallization of L-alanine *p*-chlorobenzenesulphonate (16%); the D-enantiomer underwent racemization in the liquid phase.

Dating studies continue to be supported by more detailed knowledge of the factors determining racemization rates of amino acids in their free state and bound within proteins and peptides. Fossil bones from Upper Paleolithic times, from Languedoc caves, are correctly dated on the basis of D:L ratios for their alanine and aspartic acid content only if rather higher racemization rates are assumed (based on ^{14}C dates) than those used hitherto.[224] Isoleucine isolated from fossil shells is highly epimerized before it is released from its protein sources;[225] epimerization rates for isoleucine in proteins are dependent on the position in the sequence, being fastest at the N-terminus. Similar studies have been undertaken for free and peptide-bound serine and aspartic acid, to attempt to account for the contributions of different phases through which these amino acids would have passed during ageing.[226]

General Reactions of Amino Acids. – Functional-group transformations mainly involving the $-NH_2$ and $-CO\cdot O-$ moieties of an α-amino acid are collected in this section, with the following section emphasizing the role of the side chain and transformations of functional groups present in it.

Reviews have appeared covering the electrochemistry of amino acids,[227] α-methoxylation of α-amino acids and β-lactams by electro-oxidation,[228] and reactions of oxazolinones derived from α-amino acids.[229]

Reactions at the amino group include condensation with 3,5-dinitro-1-(4-nitrophenyl)-4-pyridone to give conveniently protected derivatives[230] and cleavage of a phthaloyl group in an efficient two-stage, one-flask operation using $NaBH_4$ in 2-propanol followed by acetic acid.[231] *N*-Alkylation of *N*-Boc-amino acids with an alkyl iodide after treatment with KH and 18-crown-6 proceeds in good yield, and products with excellent optical purity can be obtained.[232] *N*-Alkylation of amino acids through condensation with an aldehyde in the

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presence of $NaBH_3CN$ in MeOH gives excellent yields[233] (for a two-stage equivalent of this process, using formaldehyde, see ref. 108). Amino acids react with formaldehyde and secondary phosphines to give *N*-phosphinomethyl derivatives $R^1PPhCH_2NHCHR^2CO_2H$.[234] *N*-Diphenylphosphinylamino acids, advocated for use in peptide synthesis, are obtained by reaction of the corresponding esters with $Ph_2P(O)Cl$.[235]

Reactions at the carboxy group to bring about conversion into other carbonyl derivatives include formation of symmetrical anhydrides and aryl esters from *N*-protected amino acids, using di-t-butyl pyrocarbonate,[236] and the formation of symmetrical anhydrides of various N-alkoxycarbonyl-L-valines through condensation with 0.5 equivalents of *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide hydrochloride in CH_2Cl_2.[237] Excess of reagent leads to the 2-alkoxyoxazolin-5(4H)-one (except for 2,2,2-trichloroethyloxycarbonylvaline) as well as the anhydride.[237] There are more conventional esterifications of the amino acids themselves achieved by adding toluene-*p*-sulphonic acid to a suspension of the amino acid in methanol or ethanol and refluxing the mixture for 24 h;[238] methyl toluene-*p*-sulphonate in refluxing methanol was used in the preparation of amino acid methyl esters.[239] N-Protected amino acids can be converted into methylthiomethyl esters using Bu^tBr and Me_2SO under mild conditions.[240] Fmoc-amino acid trichlorophenyl esters have been prepared from the N-protected amino acid and the phenol, using DCCI,[241] and *N*-Boc- or -Z-amino acid amides from the N-protected amino acids, conc. aqueous NH_4OH, and isopropyl chloroformate in THF containing *N*-methylmorpholine.[242]

Reduction of the carboxy group proceeds in high yield, *via* N-protected amino acid esters, when $NaBH_4$ in Bu^tOH with slow addition of MeOH is used.[243] Decarboxylation of N-protected amino acid *N*-hydroxypyridine-2-thione esters through photolysis in the presence of Bu^tSH (aspartic and glutamic acids also undergo reductive loss of their side-chain carboxy groups) extends the already broad applicability demonstrated for this reaction.[244]

Thermal decomposition of amino acids has been followed by thermovoltaic detection, thermogravimetry, and differential scanning calorimetry.[245] While the

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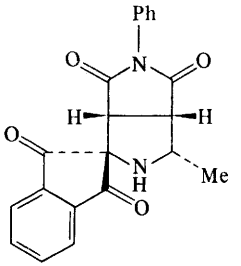
[245] S. Contarini and W. W. Wendlandt, *Thermochim. Acta*, 1983, **70**, 283.

study indicated the thermovoltaic method to be the more reproducible of the techniques, no attention was given to the nature of the degradation products.

Decarboxylative transamination is a term coined to describe the consequences of condensation of an amino acid with carbonyl compounds in the presence of a dipolarophile.[246–248] The initial condensation creates a 1,3-dipole $R^1\overset{+}{C}HN\bar{C}HR^2$ and its mesomeric equivalent from an aldehyde R^1CHO and an amino acid $\overset{+}{N}H_3CHR^2CO_2^-$ through decarboxylation; 1,3-dipolar cycloaddition then ensues if there is a suitable opportunity. A spectacular example is the formation of the pyrrolidine (13) through the reaction of ninhydrin, alanine, and *N*-phenylmaleimide.[248]



(13)

Addition of L-alanine benzyl ester to $PhCOCH{=}CHCO_2Et$ proceeds regioselectively and diastereoselectively, to give *N*-[(1*S*)-ethoxycarbonyl-3-phenylpropyl]-L-alanine after catalytic hydrogenation.[249] Other reactions involving the amino group of an α-amino acid in more unfamiliar processes are carbamoylation by *N*-nitroso-*N*-butylurea in aqueous solution (for 3 weeks),[250] polymerization of L-phenylalanine by triphenyl phosphite in a matrix support,[251] and grafting of L-amino acids onto silica pretreated with a 3-chloropropyl- or 3-aminopropyltrichloro- or -triethoxy-silane.[252] Phenyl isothiocyanate is well known for its addition to an amino group, but more vigorous reaction with an *N*-acylamino acid gives corresponding anilides,[253] while *N*-dimedonylamino acids give products of phenylthiocarbamoylation of the cyclohexenonyl moiety.[254] Lawesson's

[246] R. Grigg and H. Q. N. Gunaratne, *Tetrahedron Lett.*, 1983, **24**, 4457.
[247] R. Grigg, M. F. Aly, V. Sridharan, and S. Thianpatanagul, *J. Chem. Soc., Chem. Commun.*, 1984, 182.
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[254] M. Gomez Guillen and J. P. Garcia Martin, *An. Quim., Ser. C*, 1983, **79**, 109.

reagent yields *N*-thioacyl derivatives by mild treatment of corresponding acylamino acids, from which 1,2,4-triazines are easily formed by reaction with hydrazine.[255] Another reaction involving both amino and carboxy groups, 5-amino-2-phenyloxazolium ion formation from an *N*-benzoyl *N*-alkylamino nitrile as an unexpected product during the attempted hydrolysis by 60% $HClO_4$,[256] his precedents (Vol. 5, p. 304). Curtius rearrangement of *N*-acylamino acid hydrazides followed by addition to an alcohol gives diacylated *gem*-diaminoalkyl compounds used in retro-inverso peptide synthesis, but with troublesome side reactions in the isocyanate–alcohol reactions that have been given attention.[257]

Regeneration of amino acids from N-protected derivatives is discussed in another chapter in the context of peptide synthesis as far as common protecting groups are concerned. 2,4,6-Trinitrophenyl derivatives, however, do not fall in this category; their treatment with aqueous hydrazine at 30 °C is mild, so that fully active proteins can be recovered from trinitrophenylated materials.[258]

A crop of papers, no fewer than usual for a cull of a year's literature, deals with routine oxidation-kinetics studies of amino acids by standard oxidants.[259] Mechanistic interest is the common factor in a variety of reports of stereoselective hydrolysis of *N*-acyl- or -alkoxycarbonyl-DL-amino acid *p*-nitrophenyl esters in chiral (L-histidine-containing) micelles[260] and similar media.[261–263] In all such studies, the possibility of alternative pathways has to be recognized, and the hydrolysis of *N*-acetyl phenyl-alaninyl- or -valyl-imidazolides is much faster than for simple models, thus implicating an oxazolinone intermediate.[264] The role of the *N*-substituent, crucial in determining the ease of oxazolinone forma-

[255] T. P. Andersen, A. B. A. G. Ghattas, and S. O. Lawesson, *Tetrahedron*, 1983, **39**, 3419.
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tion, has been identified as the major factor in determining the rates of alkaline hydrolysis of *N*-substituted alanine, glycine, and β-alanine *p*-nitrophenyl esters.[265] Hydrolysis, aminolysis, and alcoholysis of Boc-glycine-activated esters have been studied, particularly the competition between hydrolysis and aminolysis.[266] Butylaminolysis of *cis*-2-hydroxycyclopentyl benzoylglycinate (a simple model for a peptidyl-tRNA) occurs some 300 times faster than the corresponding reaction with cyclopentyl benzoylglycinate, thus implicating anchimeric assistance by the hydroxy group.[267] Hydrolysis of *N*-acetylphenylalaninyl adenylate anhydride and the corresponding free amine has been followed spectrophotometrically for 10^{-5}M solutions.[268]

Other mechanistic studies deal with ozonation of amino acids,[269] decomposition of *N*-bromoalanine in aqueous solutions,[270] and protonation rates of proline in various media.[271]

Specific Reactions of Amino Acids. – The topics discussed deal mainly with the reactions undergone or facilitated by the side chains of common amino acids.

Among common aliphatic amino acids, 1-aminocyclopropanecarboxylic acid has become familiar as the archetypal alkene producer in plants; increasingly more papers are acknowledged here through representative citations[272] covering ethylene production. Formation of 1-butene in pea (*Pisum sativum*) epicotyls and in a cell-free pea epicotyl enzyme preparation is only feasible from (1*R*,2*S*)-1-amino-2-ethylcyclopropanecarboxylic acid[273] and not from other stereoisomers.

Chlorination of alanine by HOCl in dilute aqueous solutions gives acetaldehyde and acetonitrile, the latter arising from the reaction of $ClNH_2$ with the aldehyde.[274]

The mixture of DL-isoleucine and DL-alloisoleucine formed by chemical synthesis can be separated because the 1,5-naphthalenedisulphonic acid salt of DL-isoleucine is less soluble.[275] Moreover, epimerization of the alloisoleucine through heating in acetic acid in the presence of salicylaldehyde followed by separation allows up to 95% recovery of DL-isoleucine from such mixtures.[275]

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General reactions applied to specific amino acids include the following: the synthesis of fructosylglycine from glycine and D-glucose heated in MeOH for 3 h,[276] the Maillard reaction between glucose and lysine, to give mono- and di-fructosyl-lysine,[277] and decarboxylation processes (L-glutamic acid to γ-aminobutyric acid and proline to δ-aminovaleric acid by *Clostridium sordelli*,[278] phenylglycine to benzaldehyde using the coenzyme methoxatin in the presence of cetyltrimethylammonium bromide,[279] and thermal decarboxylation of γ-carboxyglutamic acid[280]). The last-mentioned paper[280] contains a useful broad coverage of the chemistry of γ-carboxyglutamic acid, including specific exchange of the γ-proton for ^3H, and the first synthesis of pyro-γ-carboxyglutamic acid (a reversible reaction with possible applicability in the estimation of the γ-carboxyglutamic acid content of proteins). Glutamic acid has been found to undergo deamination using pyridoxal phosphate and copper(II) smectite (a swelling phyllosilicate) to give ammonia and 2-ketoglutaric acid.[281]

Biosynthetic studies are not reviewed systematically; representative papers cover the conversion of threonine into glycine and aminoacetone in rat liver mitochondria[282] and the demonstration of 1,2-hydride shift from C-3 of (2*RRSS*,3*RSRS*)-[1-^{14}C,3-^{3}H]phenylalanine to the carbon atom that ultimately becomes the hydroxymethyl group of tropic acid *in vivo*.[283]

Nucleophilic reactivity towards alkylating agents of valine methylamide has been studied in view of the finding that the α-amino group shows a pK'_a of 7.65, somewhat higher than the value for a terminal *N*-valyl peptide.[284] Nucleophilic reactivity of the imidazole nitrogen atoms of N^{α}-acetylhistidine and its methylamide was studied in the same laboratory.[285] Lysine side-chain reactions feature in uncatalysed N^{ϵ}-methylation and -formylation reactions and their inhibition by excess ascorbic acid,[286] in a one-pot synthesis of N^{α}-Z-N^{ϵ}-Boc-L-lysine from L-lysine by successive masking of the α- and ε-amino groups,[287] and in a synthesis of the nitrosocarbamates $RO_2CN(NO)(CH_2)_4CH(\overset{+}{N}H_3)CO_2^-$.[288] Side-chain protected arginine suitable for solid-phase synthesis carries the 4-methoxy-2,3,6-trimethylbenzenesulphonyl group, removable by trifluoroacetic acid.[289]

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Tryptophan side-chain reactivity continues to attract attention (its photochemistry is featured in a later section), with Pictet–Spengler reactions with aldehydes giving β-carbolines.[290] Oxidative breakdown occurs under the conditions of iodination of tyrosine residues in peptides (N^{in}-formylation protects efficiently against this degradation).[291] N^{in}-Boc-L-Tryptophan formed with Boc-anhydride and 4-dimethylaminopyridine in MeCN[292] should also be a welcome new protected amino acid. Dye-sensitized photo-oxygenation of tryptophan in alkaline phosphate buffer gives the corresponding 4-hydroxypyrrolo-indole, which on air oxidation yields 5-hydroxy-*N'*-formylkynurenine.[293]

Ozonation degrades tryptophan into aspartic acid, kynurenine, and melanin through the intervention of hydroxy radicals, probably, since similar results are obtained through radiolysis and oxidation using Fenton's reagent.[294] Tryptophan radicals formed by electron transfer from azide radicals or $Br^{\bullet}$[295] can be 'repaired' by their treatment with an antioxidant.[296] Hydroxyl radicals formed by pulse radiolysis of aqueous solutions saturated with N_2O give 50% conversion of tyrosine into the radical resulting from addition *ortho* to the tyrosine hydroxy group and about 35% of the *meta* isomer.[297] Hydroxy radicals from ADP-Fe^{II}-H_2O_2 yield long-lived free radicals with proline and hydroxyproline, shown by e.p.r. studies to be nitroxyls (already known as the products of reaction with t-butyl hydroperoxide).[298] One-electron oxidation of cysteine by bromine or iodine radical anions is persuasively suggested to involve cysteinyl radical bromide or iodide complexes, respectively.[299] Oxidation of cysteine by iodine is much more complex than hitherto reported, involving I atoms, I^-, and I_3^-.[299]

Reactions based on the serine hydroxy group include that of the *O*-tosylated protected serine with a lithium diorganocuprate (see also ref. 79) to give a mixture of alkyl-substitution products and the protected dehydroalanine.[300] An *O*-→*N*-acetyl shift accompanies hydrogenolysis of *threo*-*O*-acetyl-β-phenyl-L-serine, leading to *N*-acetyl-L-phenylalanine in good yield.[301] β-Hydroxy-α-amino acid amides and Mitsunobo reagents give proline, pipecolic acid, and higher homologue derivatives, and lactams in some cases, together with ethers formed unexpectedly between two molecules of the amino acid derivative.[302]

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Reactions of sulphur-containing amino acids range from a down-to-earth electrochemical reduction for cleavage of L-cystine (over-reduction causes racemization)[303] to cyclization of *N*-acylpenicillamines with isopropyl chloroformate–NEt_3 to give D-β-thiolactones[304] and synthesis of (2*R*)- and (2*S*)-vinylglycinols CH_2=CHCH(NHR)CH_2OH from D- or L-methionine (R = Z or Boc),[305] employing standard sulphonium salt chemistry.

All the above narrative in this section has dealt with α-amino acids, on which most biological interest resides; but not all – the hydroxamic acid of γ-aminobutyric acid formed from the ethyl ester by reaction with NH_2OH possesses anticonvulsant and cardiovascular activity.[306]

Non-enzymic Models of Biochemical Processes Involving Amino Acids. – This section is not the only location for studies covered by this general title (amino acid–nucleotide interactions have been discussed in an earlier section[210] for example). Photochemical addition of amino acids to denatured calf thymus DNA,[307] homopolyribonucleotides,[308] polyuridylic acid,[309] or thymine[310] has been fully studied. Lysine and thymine react in basic solutions under irradiation at 254 nm to give 6-amino-2-(1-thyminyl)- and 2-amino-6-(1-thyminyl)-hexanoic acid.[310] This result is notable, side by side with the report[311] that tyrosine and tryptophan protect aqueous thymine from radiation damage.

Effects of Electromagnetic Radiation on Amino Acids. – Radiation-damage studies provide a linking theme from year to year for this section, and amino acids have been studied in this context for uranyl-sensitized photolysis in pressed KBr pellets,[312] for *X*-irradiated single crystals of L-asparagine hydrate (e.s.r.–e.n.d.o.r. study),[313] for radical yields of a mixture of amino acids (less than for the irradiated peptide of which the amino acids are constituents),[314] for γ-irradiated phenylalanine–glycylglycylphenylalanyl-leucine mixtures (three different adducts are formed),[315] and for ion-forming irradiation of amino acid–D-lactose mixtures (lyoluminescence as an index of the radical–oxygen and radical–radical reaction rates).[316]

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Asymmetric *X*-ray decomposition of DL-alanine or DL-phenylalanine in ^{18}O-enriched H_2O has been monitored through detecting the ^{18}O-labelled products by nuclear reactions.[317] It transpires that *X*-irradiated D-enantiomers are more sensitive ^{18}O-traps than their L-counterparts, and the significance of this will surely be followed up.

Pulse radiolysis and flash photolysis bring about one-electron oxidation of dopa, to give dopasemiquinone, that disproportionates to the quinone *en route* to melanin.[318] Photo-oxidation of dopa sensitized by haematoporphyrin, using 2,2,6,6-tetramethyl-4-piperidone-1-oxyl ('Tempone') as a convenient probe for monitoring oxygen consumption, proceeds mainly by a singlet-oxygen mechanism.[319] γ-Irradiation of aqueous solutions of amino acids in the presence of 2-methyl-2-nitrosopropane as a spin trap gives stable spin adducts that were analysed by h.p.l.c.[320–322] This useful method allows the identification of short-lived radicals, some the result of hydrated electron reactions, others from attack by the hydroxyl radical.

4′-(1-Azi-2,2,2-trifluoroethyl)phenylalanine eliminates N_2 rapidly at wavelengths longer than 300 nm, yielding a highly reactive carbene capable of OH and CH insertion reactions.[323]

The usual high level of interest in tyrosine and tryptophan continues. Trifluoroacetamide has been observed[324] to quench tryptophan fluorescence. The more subtle details of redistribution of the absorbed energy take many forms; fluorescence polarization for tyrosine and tryptophan has been linked to the thermal coefficient of frictional resistance to rotation in these molecules.[325] Several papers at a NATO Advanced Study Institute relate to the 'tryptophan problem',[326] also concerned with the decay processes available to the irradiated molecule. Laser flash photolysis of indole at 265 nm in the presence of glycine, proline, or hydroxyproline shows variations in yields of hydrated electrons, triplet-state intermediates, and indole cation radicals.[327]

A radioprotective effect has been ascribed[328] to 2-mercaptopropionylglycine against 3Gy γ-radiation.

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7 Analytical Methods

Gas–Liquid Chromatography. – The year's literature amounts to consolidation of existing methods, with *N*-heptafluorobutyroyl isobutyl esters as the most favoured derivatization objective (for analysis of D-alanine in bacterial cell walls,[329] for glycine,[330] and more generally,[331,332] especially[332] for micro-scale sampling of biological fluids). *N*-Trifluoroacetyl butyl esters[330] and pentafluoropropionyl methyl esters[333] have also been used, the latter for quantitative analysis of enantiomers of lanthionines, cystathionines, β-methyl-lanthionines, and *S*-alkyl cysteines using 'Chirasil-Val' as the chiral stationary phase.[333] Less familiar derivatization routines based on *N*-ethoxycarbonyl isopropyl esters and trimethylsilyl analogues,[334] reaction of phosgene with *N*-methylamino acids (for enantiomeric analysis using XE-60 rendered chiral with L-valyl-(*R*)-phenylethylamide as stationary phase),[335] and comparison of oxazolidinones formed between amino acids and 1,3-dichlorotetrafluoroacetone with perfluorinated derivatives for analysis at femtomole levels, have been described.[336]

Ion-exchange Chromatography. – Routine amino acid analyser regimes are not covered in this review, although indications of developments are to be found in triple-column procedures with fluorimetric quantitation,[337] appraisal of sources of error in 'high-performance' amino acid analysers,[338] and modern automated techniques for the analysis of 1- and 3-methylhistidines, tyrosine, phenylalanine, tryptophan, lysine, histidine, and arginine in urine.[339] 3-Methylhistidine features in a related study[340] (see also ref. 331 for g.l.c. analysis of 1- and 3-methylhistidines), and cation-exchange analysis of lysine–glutamic acid mixtures[341] and of threonine, alanine, proline, and aspartic acid[342] has been reported. In the last-mentioned study, use is made of the different stability constants of complexes formed between amino acids and copper(II) or zinc(II) counter-ions incorporated in the stationary phase.[342]

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Post-column ninhydrin colour formation has been compared with *o*-phthaldialdehyde fluorimetry for amino acid analysis.[343] Ninhydrin reduced with $TiCl_3$ compares favourably with the usual ninhydrin–hydrindantin cocktail in terms of reproducible colour yield.[344]

Thin-layer Chromatography. – A useful reversed-phase technique for amino acids using MeCN–0.4% trifluoroacetic acid as the mobile phase has been introduced.[345] The crosslinking amino acids desmosine, isodesmosine, merodesmosine, and lysinonorleucine are separated from each other and from all other amino acids present in elastin hydrolysates[346] by routine t.l.c. methods.

Amino acid derivatives feature prominently in the recent literature, dansyl derivatives lending themselves well to the identification of hydroxylysine through double-labelling techniques in collagen hydrolysates.[347] Reversed-phase[348] and polyamide t.l.c.[349] have been shown to offer useful advantages in the analysis of dansylamino acids, and the use of silica gel with the solvent mixtures that constitute the overpressured layer-chromatography technique has been shown to be suitable for identification of phenylthiohydantoins of the common amino acids.[350] Sarcosine has been derivatized using NBD-Cl to permit its analysis by t.l.c.[389]

High-performance Liquid Chromatography. – This has become the major separation technique for most areas of amino acid analysis, though the relative size of this section and of the preceding sections distorts the relationship since some ion-exchange results, for example, are mentioned here rather than in the 'Ion-exchange Chromatography' section earlier in this section.

Papers referring to post-column derivatization methods are covered first; of these,[351–363,17,102] several[351,352] refer to introduction of a fluorophore, through reaction of the eluate with *o*-phthaldialdehyde and a thiol, others employ electrochemical detection,[353–355] and another[358] describes the creation of fluorescent products through post-column treatment with an L-amino acid oxidase and peroxidase immobilized on aminopropylated glass beads (for the analysis of tyrosine, phenylalanine, tryptophan, and methionine). Brief details of the analytical objectives of these studies are: trimethyl-lysine,[351] collagen hydrolysates (NaOCl–*o*-phthaldialdehyde as the reagent system),[352] cysteine, homocysteine, and glutathione,[353] biogenic amines and their precursor amino

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acids,[354] phenylalanine and tyrosine in serum,[355] tryptophan[356–358] together with tyrosine,[357] dityrosine in wool hydrolysates,[359] 3-methylhistidine in urine,[360] pipecolic acid in human plasma,[361] ε-(γ-glutamyl)lysine in protein hydrolysates,[17] and (4′-amino-3′-hydroxyphenyl)alanine and (7′-hydroxybenzothiazol-4′-yl)alanine and other degradation products in phaeomelanin hydrolysates.[102] Several of these studies employ cation-exchanger phases;[352,353,360] one[362] concentrates on reversed-phase applicability, and the potential of ternary gradient systems is fully explored.[363]

Derivatives of amino acids are formed from sample mixtures prior to h.p.l.c.[364–379] (pre-column treatment). Dansylamino acids[364–367] feature in several papers, and two papers[368] continue the recent interest in dabsylamino acids. Chemiluminescence generated by post-column reaction of dansylamino acids in eluates with bis-(2,4,6-trichlorophenyl)oxalate and H_2O_2 (see also Vol. 16, p. 42) offers sensitivity that is not available in other detection methods. The separation of glutamic acid, glutamine, and γ-aminobutyric acid[366] and entantiomeric analysis of dansylated amino acids using the chiral mobile-phase technique [copper(II)acetate–*N*,*N*-di-n-propyl-L-alanine[365,367] or copper(II) aspartame[367]] are notable applications of h.p.l.c. methods. Pre-column treatment with *N*-(9-acridinyl)maleimide yields a fluorescent derivative with cystine that is conveniently separated and quantitated by h.p.l.c.[369] 9-Fluorenylmethyl chloroformate reacts with amino acids under mild conditions (within 30 s) to give stable fluorescent derivatives that can be extracted into pentane and separated by h.p.l.c.[370] *o*-Phthaldialdehyde–thiol derivatization remains popular for pre-column introduction of a fluorophore.[376,377] This crop of papers includes a novel approach to the preparation of diastereoisomeric derivatives that allow enantiomeric analysis using sensitive fluorescence assay; instead of a simple thiol

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(2-mercaptoethanol[372–375] as a general rule) *N*-acetyl-L-cysteine[376] or its Boc analogue[377] is used as the derivatization reagent with *o*-phthaldialdehyde. Other points of interest from these papers include a rapid analysis (16 min),[371] multiple-step gradient procedures,[374] and accurate determination of D-:L-aspartic acid ratio (5 picomoles of D-aspartic acid in the presence of 500 picomoles of the L-enantiometer[376]). In other derivatization procedures, 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (referred to in instrumentation manufacturers' advertisements as 'Marfey's reagent') enables the separation and determination of enantiometer ratios for amino acids by reversed-phase h.p.l.c.,[378] and the use of *N*-benzyloxycarbonylamino acids amidated with glycyl *p*-nitrophenyl-L-alanine methyl ester achieves the same objective.[379]

The conversion of amino acid mixtures into *N*-phenylthiocarbamoyl derivatives through pre-column treatment with phenyl isothiocyanate is advocated repeatedly in the recent literature[380] (see also Vol. 16, p. 41), 1 picomole sensitivity being claimed. The process is based on the coupling step of the Edman degradation, although, since the conditions appear to be the same as those yielding phenylthiohydantoins in Edman's hands,[381] the certainty of analysing phenylthiocarbamoyl amino acids rather than mixtures of these with their isomeric phenylthiohydantoins seems in doubt.

Phenylthiohydantoins continue to generate analytical interest, h.p.l.c. on octadecylsilane–Ultrasphere[382] and reversed-phase h.p.l.c. on phenylthiohydantoins of *N*-methyl amino acids[383] featuring in a number of careful studies.[382–384] Structurally related *p*-dimethylaminoazophenylthiohydantoins also feature in recent papers.[385,386] Use of a chiral derivatizing agent, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate, for generating diastereoisomeric mixtures of the resulting thiohydantoins with partly racemic amino acids has been compared for h.p.l.c. enantiomeric analysis with the use of simpler analogues but with a chiral mobile phase [copper(II) *N*-tosyl-D-phenylglycine, *cf.* refs. 365 and 367).[387]

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The foregoing paragraphs underline the growing preoccupation with assessments of both identity and enantiomeric purity of amino acid samples, and an example of a covalently bonded chiral stationary phase for the purpose, Lichrosorb-NH_2, to which an alkylaminocarbonyl-L-valine is condensed, has been evaluated.[388]

Fluorescence Methods. – Reference has been made in preceding sections to a number of common fluorogenic reagents used in conjunction with a chromatographic procedure. Imino acids do not react with *o*-phthaldialdehyde, and they can be estimated in an amino acid mixture after all amino acids have been derivatized with this reagent by treatment with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (giving derivatives with $\lambda_{emission}$ 524–540 nm).[16] The procedure has been used to assess the presence of *cis*- and *trans*-3- and -4-hydroxyprolines in collagen hydrolysates[16] and also the estimation of sarcosine (using t.l.c. for separation).[389]

Determination of Specific Amino Acids. – Colorimetric methods have been used for assays of arginine (thymol with NaOBr yields a yellow product, λ_{max} 440 nm),[390] hydroxyproline (Chloramine-T with *p*-dimethylaminobenzaldehyde gives a red product, λ_{max} 560 nm),[391] and γ-carboxyglutamic acid (red product, λ_{max} 530, with 4-diazobenzenesulphonic acid, not yielded by other amino acids).[392]

Enzymatic methods also continue to provide reliable assay procedures because of their specificity; recent examples are arginase for the conversion of arginine in serum into urea,[393] asparaginase on an ammonia-gas sensor for the estimation of asparagine,[394] and a radioenzymatic method for *S*-adenosyl-L-methionine based on doubly labelled melatonin formation.[395] A spectacular example of things to come, perhaps, is the use of a column of immobilized exopeptidase as an inlet to a thermospray mass-spectrometry ion source, for the analysis of amino acids released sequentially from the C-terminus of a peptide.[396]

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