Amino Acids and Peptides

G. C. BARRETT

AND

D. T. ELMORE

CAMBRIDGE UNIVERSITY PRESS

PUBLISHED BY THE PRESS SYNDICATE OF THE UNIVERSITY OF CAMBRIDGE
The Pitt Building, Trumpington Street, Cambridge CB2 1RP, United Kingdom

CAMBRIDGE UNIVERSITY PRESS
The Edinburgh Building, Cambridge CB2 2RU, United Kingdom
40 West 20th Street, New York, NY 10011-4211, USA
10 Stamford Road, Oakleigh, Melbourne 3166, Australia

First published 1998

Printed in the United Kingdom at the University Press, Cambridge

Typeset in Times NR 10/13pt [SE]

A catalogue record for this book is available from the British Library

ISBN 0 521 46292 4 hardback
ISBN 0 521 46827 2 paperback

6

Synthesis of amino acids

6.1 General

There is an abundant supply of L-enantiomers of most of the coded amino acids. These are made available through large-scale fermentative production in most cases, and also through processing of protein hydrolysates. The early sections of this chapter cover this aspect, However, laboratory synthesis methods are required for the provision of most of the other natural amino acids and for all other amino acids, so the main part of this chapter deals with established syntheses.

6.2 Commercial and research uses for amino acids

In addition to the provision of supplies of common amino acids, there are growing needs for routes to new amino acids, since pharmaceutically useful compounds of this class continue to be discovered, which must be free from toxic impurities and homochirally pure in this particular context. Important functions for close analogues of coded and other biologically significant amino acids include *enzyme inhibition* and retarding the growth of undesirable organisms (fungistatic, antibiotic and other physiological properties, possessed either by the free amino acids or by peptides containing them). Free amino acids that perform in this way are α-amino isobutyric acid (an example of an α-methylated analogue of a coded amino acid), which has been proposed for the control of domestic wood-rotting fungi), and α-methyl-Dopa (α-methyl-3′,4′-dihydroxy-L-phenylalanine), a well-known treatment for Parkinson's disease. Similar success for new therapeutic amino acids, based on their enzyme-inhibition properties, is indicated for amino acids with a minimal structural change such as the substitution of a side-chain hydrogen atom by a fluorine atom.

6.3 Biosynthesis: isolation of amino acids from natural sources

Many examples of the discovery and isolation of amino acids from natural sources date from the early 1900s, though some were characterised several years before that (Greenstein and Winitz, 1961). Further new examples continue to be discovered, either as constituents of proteins, revealing new post-translational processes for higher organisms (Table 1.3 in Chapter 1), or in the free or bound form (from fungal or bacterial sources or from marine organisms).

6.3.1 Isolation of amino acids from proteins

Hydrolysis of proteins and separation of the resulting mixture is an obvious, and traditional way (Greenstein and Winitz, 1961) of obtaining moderate quantities of the coded and post-translationally modified L-α-amino acids. However, because of the availability of viable methods of industrial synthesis, hydrolysis of proteins no longer offers a sensible approach owing to its tedious and expensive nature and the fact that some amino acids are destroyed in the process (see Chapter 3).

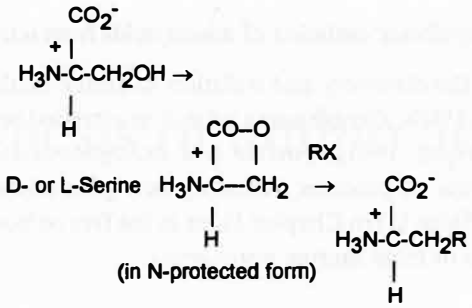
6.3.2 Biotechnological and industrial synthesis of coded amino acids

Knowledge gained of biosynthetic routes to L-α-amino acids and isolation of the enzymes mediating the steps in these routes has been exploited for the industrial-scale manufacture of most of the coded L-α-amino acids. In some cases, the enzymatic production of near-analogues of the coded L-α-amino acids can also be achieved (Goldberg and Williams, 1991; Rozzell and Wagner, 1992).

To illustrate the methods, a culture medium that contains indole, pyruvic acid, tyrosine phenollyase and an ammonium salt, as well as the usual buffers and salts, will accumulate L-tryptophan; or will produce an indole-substituted L-tryptophan if indole itself is replaced by a substituted indole. L-Dopa formed in a system employing tyrosinase from *Aspergillus terreus* provides a further example of this approach (Chattopadhyay and Das, 1990).

The crucial enzymes need not be isolated, since 'bio-reactors' containing micro-organisms that are fed with the appropriate starting materials are often more convenient. L-Threonine from *Brevibacterium flavum*, L-lysine from *Corynebacterium glutamicum* (Eggeling, 1994) and use of plant-cell suspension cultures illustrated by L-Dopa from *Mucuna pruriens* (Wichers *et al.*, 1985) are examples. However, bio-engineering of the whole organisms to be used in this way may need to be carefully optimised to achieve reasonable yields. The other main opportunity offered by bio-technological methods is the conversion of one amino acid into a less plentifully available amino acid, e.g. the conversion of L-tyrosine into L-Dopa using *Mucuna pruriens* (Wichers *et al.*, 1985).

For a limited range of amino acids, this approach is increasingly in competition



Scheme 6.1.

with chemical synthesis, which can accomplish the necessary modifications in some cases more easily (Section 6.4). Examples of 'non-biotechnological' synthesis are provided by the industrial production of glutamic acid and lysine, conducted on a large scale (several thousand tons per year). DL-Glutamic acid is obtained from acrylonitrile, electrochemical reductive dimerisation and functional group modifications giving the DL compound. DL-Lysine is obtained from caprolactam, through its 3-amino-derivative, which is resolved (Scheme 6.6) with L-pyroglutamic acid before ring-opening to give L-lysine.

6.4 Synthesis of amino acids starting from coded amino acids other than glycine

With the easy availability of many of the natural amino acids, some general methods for the synthesis of more complex structures are based on the modification of simple natural amino acids. An important benefit from this approach is the fact that homochirality at the α-carbon atom can be preserved in reactions at side-chains that are in current use.

Thus, D- or L-serine can be converted through the Mitsunobu reaction into the homochiral α-amino-β-lactone, a chiral synthon amenable to ring-opening by organometallic reagents (Pansare and Vederas, 1989) to give β-substituted alanines (Scheme 6.1). β-Iodo-L-alanine (also obtained from L-serine) can be elaborated similarly into the general class of β-substituted alanines (L-serine → $H_3N^+CH(CH_2I)CO_2^- \rightarrow H_3N^+CH(CH_2R)CO_2^-$ (Jackson *et al.*, 1989)). L-Aspartic acid and L-glutamic acid serve the same function, electrophiles being substituted at the carbon atom next to the side-chain carboxy group after its deprotonation with lithium di-isopropylamide (Baldwin *et al.*, 1989). As shown in this composite example from a number of research papers, the side-chain carboxy group can be transformed into other functional groups, when one starts with suitably protected glutamates and aspartates (Scheme 6.2).

There are numerous other isolated examples of the conversion of a coded amino acid into another amino acid. These usually amount to applications of straight-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.