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ENZYMIC SYNTHESIS OF ISOTOPICALLY LABELLED L-TYROSINE

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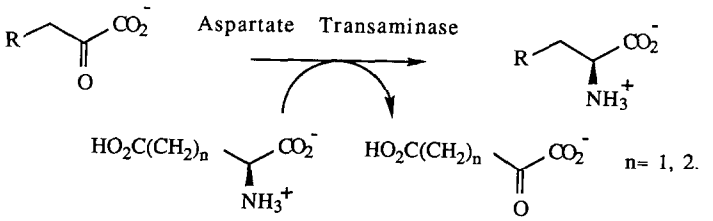
Abstract: L-Tyrosine specifically labelled with ^{13}C and ^{15}N has been enzymatically synthesised from achiral precursors in high yield and optical purity.

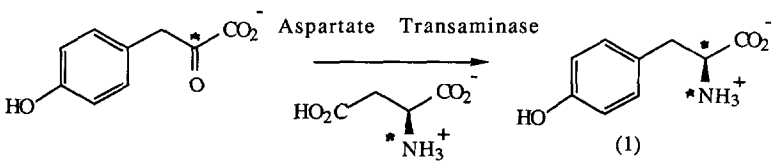
Stable isotope-labelled amino acids are required for a variety of bioorganic chemical studies[1]. Such studies are generally limited by the cost and availability of appropriately labelled compounds. We have recently described an efficient enzymatic synthesis of L-α-amino acids (Scheme 1)[2]. We herein report that this methodology provides a facile, economical synthesis of nmr active, isotopically labelled L-α-amino acids as illustrated by the synthesis of ^{13}C and ^{15}N labelled L-Tyrosine.

Scheme 1



^{15}N Labelled Tyrosine (1b) (>95%ee) was prepared in 80% yield by transamination of (4-Hydroxyphenyl)pyruvic acid with ^{15}N-DL-Aspartic acid*, catalysed by purified[3], recombinant[4] *E. coli* Aspartate transaminase (Equation 1). Enzymatic reactions have been used to introduce the requisite stereochemistry in previous *in vivo* "chemobiological" syntheses of L-Tyrosine[5]. The *in vitro* method reported here, avoids whole cells and corresponding requirement for microbiological techniques. This simplified preparation and purification also ensures very high levels of isotopic incorporation (>95%).

Equation 1

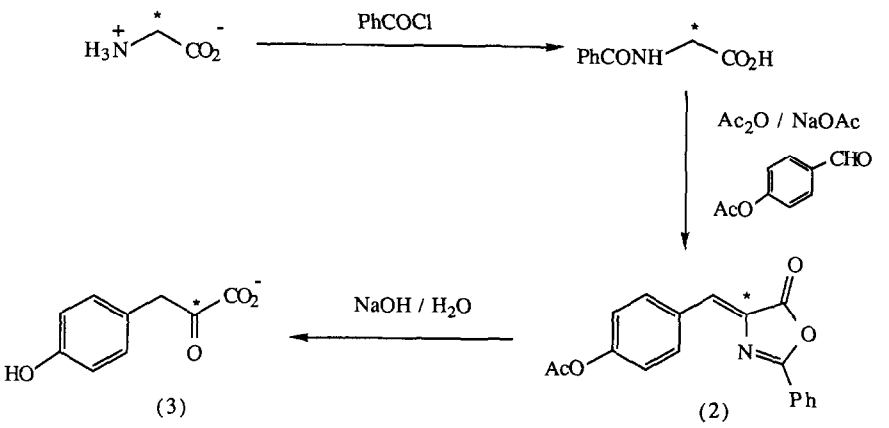


(1)

[a; ^{12}C, ^{14}N, b; ^{12}C, ^{15}N
c; ^{13}C, ^{14}N, d; ^{13}C, ^{15}N]

The synthesis of [2-^{13}C]-L-Tyrosine utilised [2-^{13}C]-Glycine as the label source. [2-^{13}C]-Glycine was benzoylated then condensed with 4-Acetoxybenzaldehyde to give azlactone (2) (60%, 2 steps). Hydrolysis to [2-^{13}C]-(4-Hydroxyphenyl)pyruvic acid (Equation 2), and transamination of crude α-keto acid (3) using L-Aspartic acid as nitrogen donor provided [2-^{13}C]-L-Tyrosine (2c) in 60% yield from azlactone (2).

Equation 2



Combination of the above pathways provides a facile route to [^{15}N, 2-^{13}C] amino acids. Such compounds are of great utility in biosynthetic studies for identifying whether such CN linkages remain intact during in vivo transformations. Using ^{15}N-DL-Aspartic acid as the nitrogen donor in the transamination reaction with [2-^{13}C]-(4-Hydroxyphenyl)pyruvic acid, [^{15}N, 2-^{13}C]-L-Tyrosine (2d) was obtained directly in an identical fashion to the other isotopomers (Equation 1). This compound is currently being used to identify the biosynthetic origin of the isonitrile nitrogen of Trichoviridin and related metabolites[6].

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* Whilst the enzyme only utilises L-amino acids, no problems are encountered on using the more readily available DL amino acids as substrates.

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