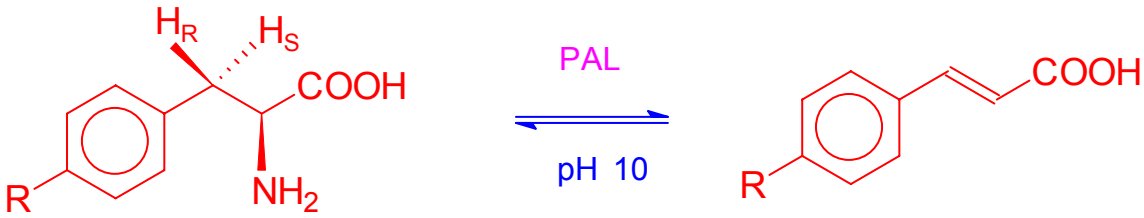
ABSTRACT

L-Tyrosine (L-Tyr) doubly labeled with ^{14}C and with deuterium or tritium in 3-R and 3-S positions was synthesized using specific properties of the following enzymes: PAL (Phenylalanine Ammonia-Lyase, E.C. 4.3.5.1.) and rat liver L-phenylalanine hydroxylase (E.C. 1.14.16.1.). The synthesis of selectively labeled L-Tyr via cinnamic acid and L-phenylalanine (L-Phe) applying combined chemical and enzymatic methods has been elaborated. Potassium [^{14}C]-cyanide, heavy and tritiated water have been used as the sources of stable or radioactive labels. In the first step L-Phe labeled with carbon-14 was obtained by addition of ammonia to (E)-[1-^{14}C]-cinnamic acid using PAL as a catalyst. Next, L-Phe was enzymatically converted into doubly labeled L-Tyr (with deuterium or tritium in 3-S and 3-R positions and with carbon ^{14}C in the carboxylic group) applying L-phenylalanine hydroxylase activity. Yields of obtained isotopomers of L-Tyr have been determined by enzymatic and radiochemical methods.

INTRODUCTION

An enzyme PAL catalyzes elimination of ammonia and *pro-3S*-hydrogen from L-Phe leading to the formation of (*E*)-cinnamate:

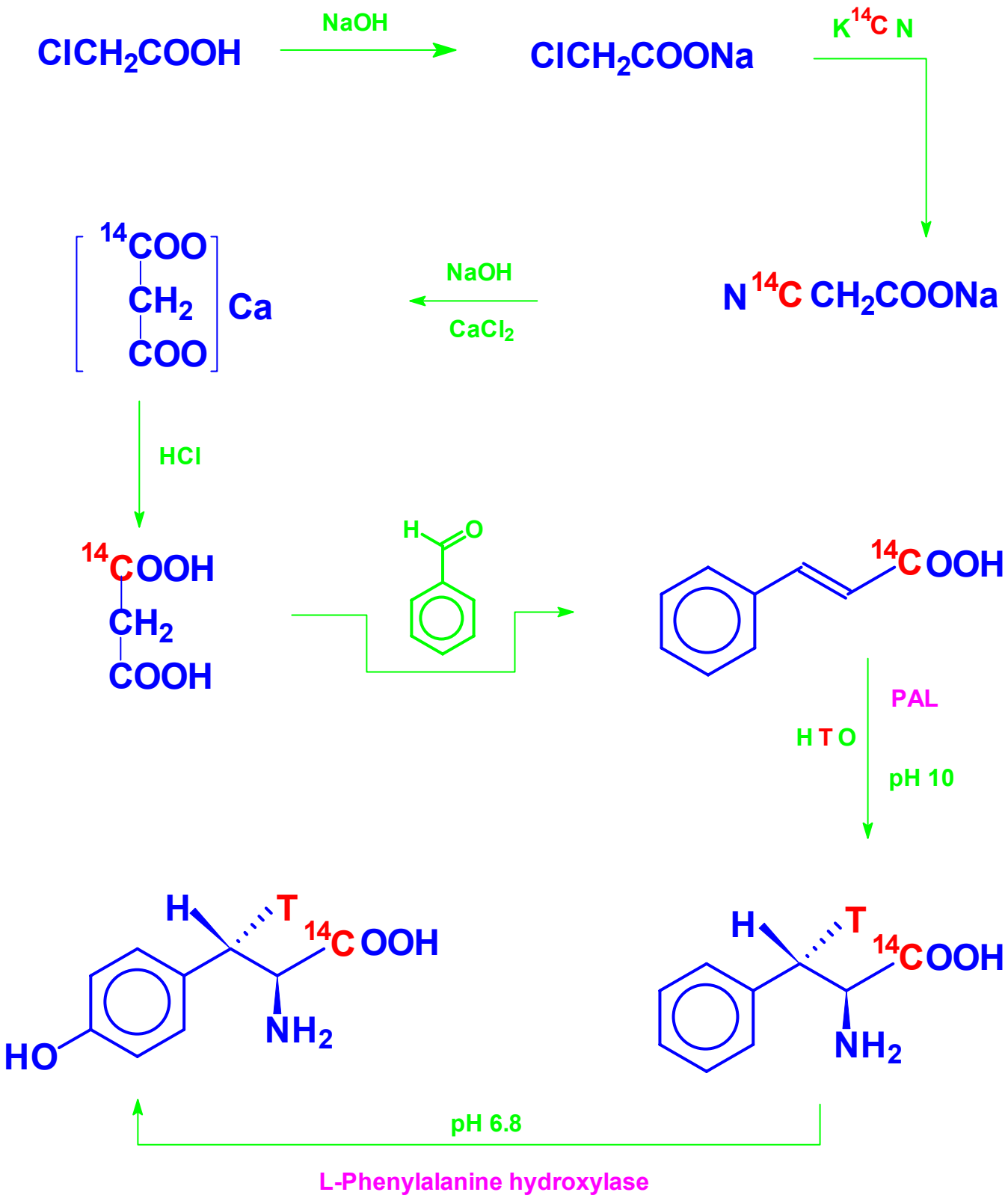


The above reaction is a multistep process involving several intermediates and therefore is important to determine the structure of active complex formed during the rate determining step. There are several literature reports on the PAL including the structure of its active center and enzyme inhibitors. The proposed mechanism of this reaction and the some currently unclear intrinsic details have also been reported [1-4]. The enzyme PAL catalyzes elimination of ammonia from L-tyrosine (L-Tyr) and *O*-methyl -L-Tyr [5].

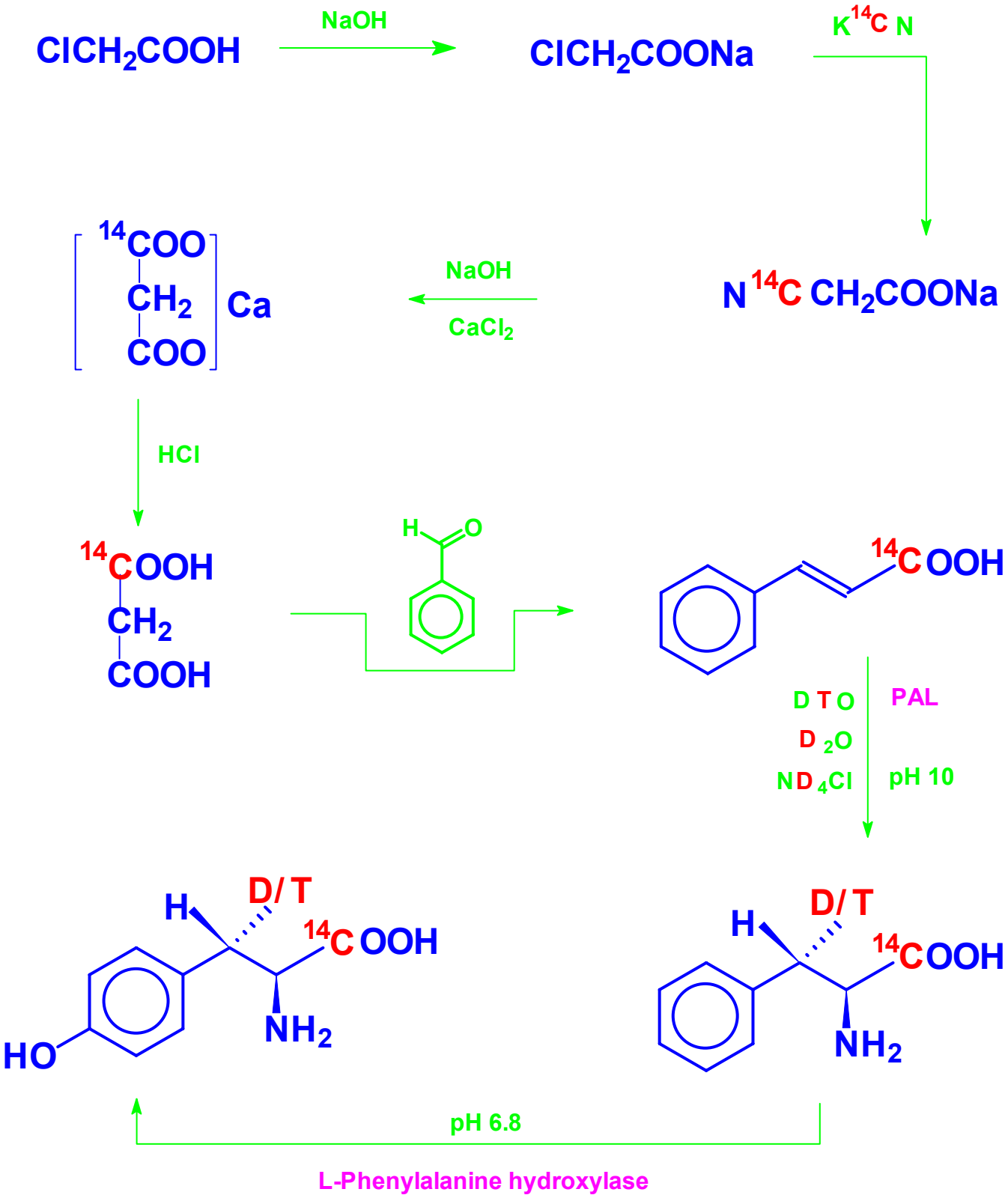
The goal of our research is to investigate the mechanism of this reaction with kinetic isotope effect (KIE) method [6]. Numerical values of deuterium and tritium KIEs on the bonds directly involved in chemical reaction allow to discover the tunneling effect [7] in a given reaction according to the Saunders criterion [8]. The atoms taking part in the ammonia elimination from L-Phe derivatives of shall be labeled with radioactive or stable isotope. It is possible to find out the number of reactive atoms and to elucidate the structure of the transition state by determination of primary and secondary deuterium and tritium KIE. The presence of *para* substituents in the ring would allow to investigate the effect of donor-acceptor abilities of these groups on the reaction mechanism.

The aforementioned studies require the synthesis of L-Phe derivatives e.g. L-tyrosine (L-Tyr) doubly labeled with deuterium tritium in 3*S* and 3*R* position of side chain and with carbon-14 in the carboxylic group. The ^{14}C label in carboxylic group will serve as an internal radiometrical standard during hydrogen KIE determination.

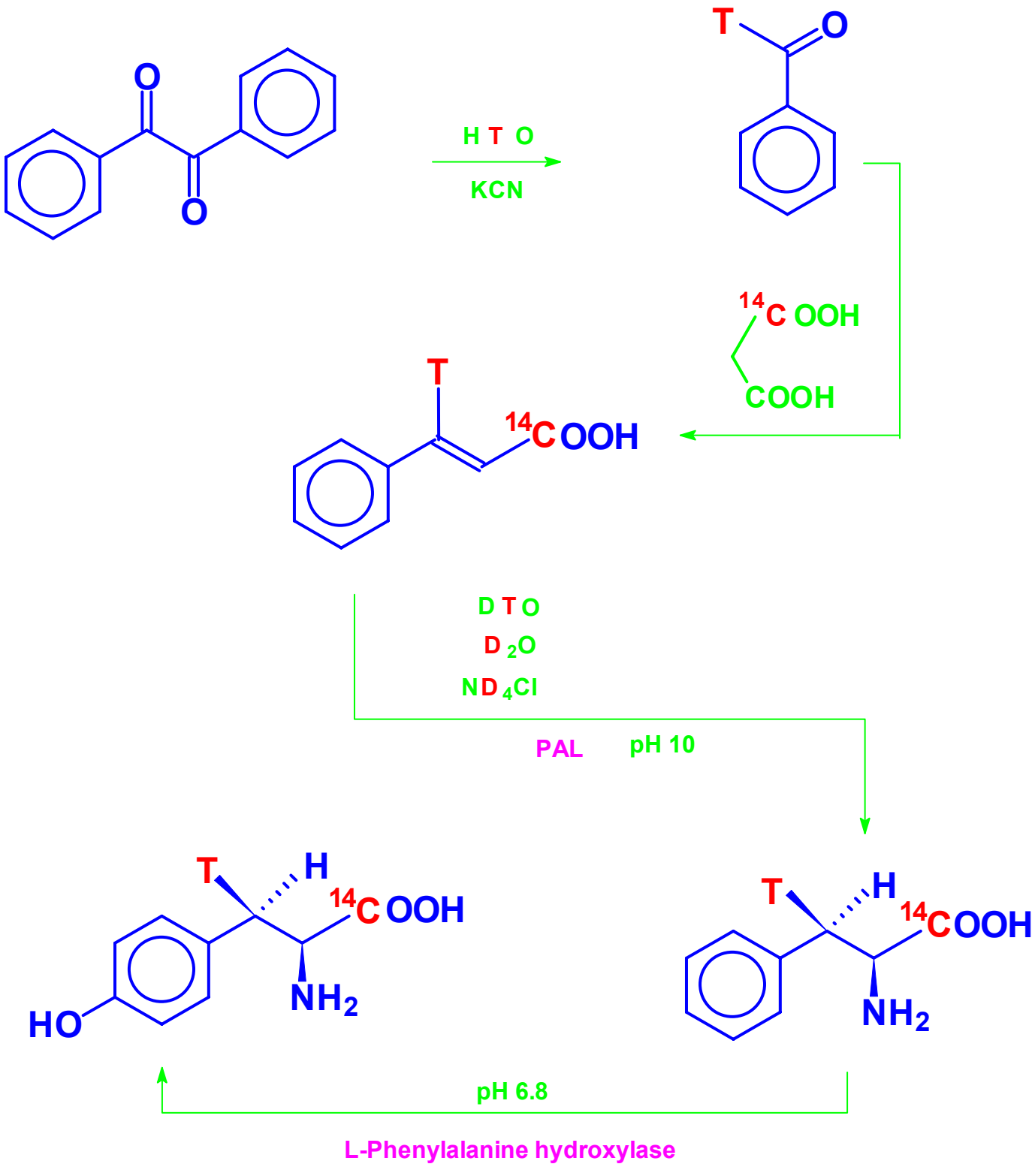
SYNTHESIS OF [3*S*-^{3}H]-[1-^{14}C]-L-Tyr



SYNTHESIS OF [3*S*-^{3}H/^{2}H]-[1-^{14}C]-L-Tyr



SYNTHESIS OF [3*R*-^{3}H]-[1-^{14}C]-L-Tyr



CONCLUSIONS

The synthesis of some labeled intermediates were elaborated earlier in our group [9,10]. All the final products were purified by column chromatography and preparative TLC. The purity of obtained amino acids was proven with TLC and enzymatic methods. The radiochemical yields of reactions were determined by liquid scintillation counting. The chemical yields have been determined by enzymatic and spectral methods.

We are elaborating the synthesis of other multilabeled isotopomers of L-Tyr. Currently, we are preparing the deuteriated substrate ([3-^{2}H]-cinnamic acid) and cinnamic acid labeled with ^{14}C in various positions of the side chain as the precursors for the synthesis of various isotopomers of L-Tyr.

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