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Synthesis and Purification of ^{11}C-Carboxyl-labeled Amino Acids

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Introduction

AS THE result of recent advances in nuclear medical instrumentation, i.e. positron computerized axial tomography,[1] the use of ^{11}C ($T_{1/2}$ = 20.4 min) in medical diagnosis has received renewed attention.

We have recently undertaken the synthesis of a number of ^{11}C-labeled amino acids for use in our nuclear medicine clinical research program for the detection of cancer and pancreatic disease. Although other workers had reported that the Strecker technique gave nonreproducible results and low yields of amino acids in reaction times compatible with ^{11}C labeling,[2,3] we reasoned that, with large amounts of ^{11}C activity, clinically useful levels of ^{11}C-carboxyl-labeled amino acids might still be produced by suitable modifications of the Bücherer–Strecker technique.

In a Strecker-type synthesis of a ^{11}C-labeled amino acid, the yield with respect to cyanide will necessarily take precedence over yield with respect to the organic starting material, and a high rate of reaction is obviously desirable because of the short half-life of ^{11}C. Therefore, in our first attempts at the synthesis of one of these amino acids, ^{11}C-carboxyl-labeled 1-aminocyclopentanecarboxylic acid (^{11}C-ACPC, a tumor-localizing agent), we investigated not only the use of an excess of the organic starting material (cyclopentanone) but also the effects of increases in reaction temperature and pressure. It now appears from our further investigations that the method developed for production of ^{11}C-ACPC[4] is a generally successful one for the rapid synthesis and purification of other ^{11}C-carboxyl-labeled neutral amino acids, and also that, with certain modifications, it is applicable to the synthesis of a ^{11}C-carboxyl-labeled basic amino acid, ^{11}C-DL-tryptophan.

Procedures

^{11}C-*Carboxyl-labeled neutral amino acids*

The previously reported details of the two-step synthesis and ion-exchange purification of ^{11}C-ACPC[4] apply to the other neutral amino acids reported in this note. Quantities of reagents used were the following: the appropriate carbonyl compound, 0.5 mM; $(NH_4)_2CO_3$, 0.75 mM; NH_4Cl, 0.125 mM; and KCN, 0.125 mM. The 210°C reaction temperature used is readily obtained by insertion of the reaction vessel into a large heated metal block. Rapid liquid flow through the ion-exchange beds used in purifications is obtained by a positive air pressure of 5 to 7 psi.

^{11}C-*Carboxyl-labeled* DL-*tryptophan*

The synthesis and purification of the basic amino acid, ^{11}C-carboxyl-labeled DL-tryptophan, required some alterations in the procedure used for neutral amino acids: The instability of 3-indoleacetaldehyde to either acid or base necessitated the use of the 3-indoleacetaldehyde bisulfite addition compound. Also tar formation, which caused processing difficulties when an excess of this carbonyl derivative was used, led to a change in the relative quantities of the reagents used, i.e. 3-indoleacetaldehyde bisulfite addition compound, 0.1 mM; $(NH_4)_2CO_3$, 0.6 mM; NH_4Cl, 0.1 mM; and KCN, 0.1 mM. Finally it was necessary to modify the first step of the purification procedure by the use of a column of Porapak Q (Waters Associates, Inc., Milford, Mass.) in place of the anion-exchange resin used with neutral amino acids. The cooled reaction mixture is filtered, acidified with 2 ml of 6 N HCl, and then loaded onto a 1.0 × 10-cm column containing Porapak Q, 80–100 mesh. (The column is prepared beforehand by soaking the Porapak Q in acetone, loading the column, rinsing with 40 ml of water per gram of Porapak Q, and finally rinsing well with 0.1 N HCl.) The loaded column is washed twice with 0.1 N HCl, once with water and finally eluted with 50% aqueous ethanol. The eluate is acidified with 2 ml of 6 N HCl (already present in the receiver) and then loaded directly onto a prewashed cation-exchange bed as in the purification of neutral amino acids.

The time required for synthesis and purification of ^{11}C-carboxyl-labeled amino acids by our method is approximately 45 min. Before they are used clinically, however, a final radiopharmaceutical processing is required (20–25 min). This consists of neutralization, microfiltration, and a rapid (15 min) check for pyrogens[5] using the Pyrotest Limulus amebocyte lysate technique (Difco Laboratories, Detroit, Mich.).

Results

Table 1 lists the percent chemical yields of the synthesized and purified amino acids (based on the stable cyanide used) and the maximum ^{11}C-activity levels that have been achieved to date for ^{11}C-ACPC and a number of other ^{11}C-carboxyl-labeled amino acids. The synthesis time in each case was 10 min for each of the two steps. Values given for the percent chemical yield (column 3) are a much better relative indication of the achievable ^{11}C-activity yields than the values given in the last column of Table 1, since there were substantial variations in the ^{11}C target

TABLE 1. Production of various purified amino acids using a high-temperature, high-pressure modification of the Bücherer–Strecker technique

Amino acid*	Precursor carbonyl compound	Chemical yield† (%)	Maximum yield of ^{11}C-labeled compound achieved to date (mCi)
1-Aminocyclopentane-carboxylic acid (ACPC)	Cyclopentanone	40	171
1-Aminocyclobutane-carboxylic acid	Cyclobutanone	55	136
1-Aminocyclohexane-carboxylic acid	Cyclohexanone	55	75
1-Amino-2-methylcyclo-pentanecarboxylic acid	2-Methylcyclopentanone	45	—
1-Amino-3-methylcylco-pentanecarboxylic acid	3-Methylcylcopentanone	48	—
DL-Valine	Isobutyraldehyde	70	363
DL-Tryptophan	3-Indoleacetaldehyde bisulfite addition compound	30	63
DL-Leucine	Isovaleraldehyde	45	—
DL-α-Methylvaline	3-Methyl-2-butanone	8	—

* The first 5 amino acids show affinities for malignant tissue; the next 4 show affinities for normal pancreas.
† Based on amount of stable cyanide used; see text for ratios of reactants.

performance and in the conversion of $^{11}CO_2/^{11}CO$ to $H^{11}CN$[4], which were undergoing developmental changes during the time that these syntheses were performed. For DL-leucine, DL-α-methylvaline, 1-amino-2-methylcyclopentanecarboxylic acid and 1-amino-3-methylcyclopentanecarboxylic acid, the yields reported are based on the synthesis of the ^{14}C-labeled amino acids; the corresponding ^{11}C-labeled compounds were not synthesized, because ^{14}C animal tissue distribution studies indicated that they probably would not be sufficiently promising as either cancer- or pancreas-imaging agents when labeled with ^{11}C. The low chemical yield for DL-α-methylvaline is probably attributable to steric hindrance.

Summary

A rapid high-temperature modification of the Bücherer–Strecker amino acid synthesis, developed for the production of ^{11}C-carboxyl-labeled ACPC, has been found to be of general use for the synthesis of other ^{11}C-labeled neutral amino acids, and, with certain modifications, was also found to be applicable to the synthesis of ^{11}C-labeled DL-tryptophan, a basic amino acid. The method requires 20 min for synthesis and an additional 25 min for purification. Chemical yields of the purified amino acids, based on cyanide, have ranged from 10–70%; ^{11}C activity levels as high as 363 mCi (^{11}C-DL-valine) have been obtained in the final product.

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