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HIGH-LEVEL PRODUCTION OF C-11-CARBOXYL-LABELED AMINO ACIDS

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ABSTRACT

C-11-Labeled amino acids have significant potential as agents for positron tomographic functional imaging. We have developed a rapid, high-temperature, high-pressure modification of the Bücherer-Strecker amino acid synthesis and found it to be quite general for the production of C-11-carboxyl-labeled neutral amino acids. Production of C-11-carboxyl-labeled DL-tryptophan requires certain modifications in the procedure. Twelve different amino acids have been produced to date by this technique. Synthesis and chromatographic purification require approximately 40 min, and C-11-carboxyl-labeled amino acids have been produced in yields of up to 425 mCi. Two C-11-carboxyl-labeled amino acids are being investigated clinically for tumor scanning and two others for pancreatic imaging. Over 120 batches of the various agents have been produced for clinical use over a three-year period.

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INTRODUCTION

C-11-Carboxyl-labeled amino acids ($T_{1/2}$ C-11 = 20.4 min) have recently been shown to have significant potential in nuclear medicine. [Carboxyl- ^{11}C] DL-tryptophan (1,2) and DL-valine (2,3) are useful for pancreatic imaging, and [carboxyl- ^{11}C] 1-aminocyclopentanecarboxylic acid (ACPC) (4,5) and 1-aminocyclobutanecarboxylic acid (ACBC) (6) are tumor-localizing agents. The great importance of amino acids in biochemical pathways suggests that C-11-carboxyl-labeled amino acids may have untapped potential for study of a multitude of physiological processes. Quantitative functional imaging with C-11-carboxyl-labeled amino acids is made possible by their suitability for positron computerized transaxial tomography (7).

We have developed a rapid, high-temperature, high-pressure modification of the Bücherer-Strecker amino acid synthesis and applied it to the synthesis and purification of a number of C-11-carboxyl-labeled amino acids (8). The objectives of this paper are to report some recent refinements in our high-level synthetic technique and to provide previously unreported experimental details.

TARGETRY AND PRODUCTION OF C-11 OXIDES

We use the Oak Ridge National Laboratory's 86-Inch Cyclotron for production of C-11. The combination of high beam currents (typically 150-175 μA) of 22 MeV protons with a 5 mm orbital separation distance permits the use of high-production internal-beam targets. A mixture of ^{11}CO and $^{11}\text{CO}_2$ is produced by the $^{11}\text{B}(\text{p},\text{n})^{11}\text{C}$ reaction. Figure 1 shows a cutaway view of our boron oxide (B_2O_3) target which we bombard with 22 MeV protons to produce C-11.

The B_2O_3 target is prepared as follows. B_2O_3 rods are prepared by heating B_2O_3 (m.p. 450°C) in a platinum crucible at 800°C for 30 min to remove any water present, cooling to $\sim 600^\circ\text{C}$, and drawing out strands of the material which rapidly harden upon cooling. Sections 60 mm long are then cut from those strands which have diameters of approximately 1 mm. These are stored in a closed glass tube containing Drierite until used for target preparation. A "boat" of 0.005-in.-thick Grafoil (Fiber Materials, Inc., Biddeford, ME) is formed in a mold having the same dimensions as the capsule tube (see below), to ensure a good fit of the completed target in the capsule tube. The "boat" is loaded with rods of B_2O_3 , placed in a quartz tube, and heated at 800°C for 15 min in a tube furnace (Lindberg Type 123-1, 422-watt, Lindberg, Inc., Watertown, WI, or equivalent) regulated with a variable transformer. In order to minimize trapped air, the target is fused in an atmosphere of helium or nitrogen. Since B_2O_3 shrinks when fused, this procedure may be repeated several times to achieve the desired weight of B_2O_3 in the target, i.e., approximately 0.65 g.

The B_2O_3 and its support are contained in an aluminum capsule tube of 5 mm O.D. which is in turn contained in an aluminum jacket tube of 6.3 mm O.D. Between the two aluminum tubes is an annular space which carries 15 l/min of cooling water (Fig. 1). The 60 mm wide by 6 mm high proton beam is centered on the axis of the tube. The proton energy is attenuated from 22 MeV to 16 MeV at the target midplane in passing through the jacket

wall, water annulus, and capsule wall. Air in the target and connecting tubing is removed by purging with helium and a static helium atmosphere is maintained during bombardment. The B_2O_3 is liquefied by the beam and the C-11 produced by the $^{11}B(p,n)^{11}C$ reaction combines with oxygen in the target. After bombardment it is essential that the molten B_2O_3 solidify (requiring about 30 sec in our case) to prevent the sweep gas from pushing it out of the beam's path. The resulting mixture of ^{11}CO and $^{11}CO_2$ is then swept from the target by 100 ml/min of helium to a remote control manipulator cell located adjacent to the cyclotron, where the bolus of C-11 activity is measured using an ion chamber (Fig. 2). Visual examination of several targets in a glove box after use has shown that, during bombardment, the molten B_2O_3 coats the inside of the capsule tube, facilitating good release of the mixture of C-11 oxides. Production of $^{11}CO/^{11}CO_2$ using this targetry has given a maximum yield of 5 Ci and a typical yield of 3-4 Ci for a 40-min bombardment at 150-175 μA .

No statistically significant advantage was observed in using B_2O_3 enriched in B-11 (98.4% B-11, made from enriched H_3BO_3 by heating at 800°C for 30 min) versus natural B_2O_3 (80.4% B-11) as a target material. The reason for this is unclear but may be related to the chemical form and/or chemical purity of the respective preparations. We use natural B_2O_3 (Ultrex, J. T. Baker Chemical Co., Phillipsburg, NJ) for our routine target fabrication.

CATALYTIC PRODUCTION OF $H^{11}CN$

$^{11}CH_4$ and $H^{11}CN$ are produced sequentially from $^{11}CO/^{11}CO_2$ by modifications of the catalytic methods of Bánfi et al (9). The 100 cc/min helium stream containing $^{11}CO/^{11}CO_2$ is mixed with 50 cc/min of hydrogen and passed through a quartz tube containing nickel on kieselguhr (Ni-3226, E 1/16, Harshaw Chemical Co., Cleveland, OH) (Fig. 2). The minimum path length of the catalyst is 5 cm, and it is held in place by a plug of nickel wire at each end. The quartz tube containing the nickel catalyst is first heated to 520°C in a hydrogen atmosphere using a regulated tube furnace (see above) in order to activate the catalyst, and the temperature is then lowered to 400°C and held at that level for the run. The nickel catalyst is no longer effective after it has cooled to room temperature and, therefore, is replaced after each day's run.

The output gas containing $^{11}CH_4$ is passed through a $CaSO_4/NaOH$ trap to remove water and any unreacted $^{11}CO_2$. The gas stream is then mixed with 50 cc/min of anhydrous ammonia and passed through another quartz tube containing platinum catalyst held at 950°C using a tube furnace as above. The platinum catalyst is prepared from platinum screen by cleaning and etching with aqua regia, plating with platinum black, and crumpling into small wads to increase the surface area. The path length of the platinum catalyst is 5 cm, and it is prevented from moving downstream by a constriction in the quartz tube. The $H^{11}CN$ -containing output gas from the platinum catalyst is finally bubbled into 1 ml of 0.005 N NaOH in a glass gas absorption column (0.7 cm x 25 cm) having a bulbed area near the top to enhance break-up of the bubbles (see Fig. 2).

We found that the catalytic conversion yield was always significantly lower for the first run than for subsequent runs on a given day. This was apparently due to release of water from the target which decreased the

efficiency of the nickel catalyst. This problem can be circumvented by carrying out a 5-min bombardment and venting the resulting gas stream around the two catalysts. The trapped [^{11}C] cyanide is measured in an ion chamber after it is drained into a stainless steel reaction vessel (see below). The average combined efficiency for catalytic conversion from $^{11}\text{CO}/^{11}\text{CO}_2$ and trapping of H^{11}CN is approximately 70%.

SYNTHESIS OF C-11-CARBOXYL-LABELED AMINO ACIDS

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A stainless steel reaction vessel fitted with a needle valve (Fig. 4) is used for the high-temperature, high-pressure Bücherer-Strecker amino acid synthesis. The reaction vessel is fabricated from type 304 stainless steel using a 1 7/8-in. piece of standard 3/4-in. hexagonal stock. The vessel is formed by drilling a 7/16-in. diameter hole in the stock and fitting the top with standard 1/4-in. female N.P.T. threads. The reaction vessel has a concave bottom to facilitate complete removal of the reaction mixture. The internal volume beneath the threads is 3 ml.

The needle valve has 1/4-in. male N.P.T. threads which are wrapped with 1/4-in. Teflon tape before tightening to ensure a good seal with the reaction vessel. The valve's closure is based on metal-to-metal contact between a 6-32 stainless steel screw and the needle inlet, which has a depression to permit better seating of the screw. The screw has a 3/16-in. x 2-in. stainless steel rod welded to the top to enable the valve to be opened and closed using manipulators. The entire needle assembly is hinged to swing at a right angle with the body so that liquids can easily be added to or withdrawn from the reaction vessel using a needle and syringe.

Before starting a C-11-carboxyl-labeled amino acid production run, the reaction vessel is loaded with the required reagents. For neutral amino acids, the reagents are: the appropriate carbonyl compound, 0.5 mmole; $(\text{NH}_4)_2\text{CO}_3$, 0.75 mmole; NH_4Cl , 0.125 mmole; and KCN, 0.125 mmole. In the case of [carboxyl- ^{11}C] DL-tryptophan, some modifications in the procedure are necessary. An aldehyde derivative, 3-indoleacetaldehyde bisulfite addition compound, is used because of the instability of the free aldehyde to either acid or base. In addition, tar formation caused processing difficulties when an excess of the carbonyl derivative was used and led to use of the following quantities of reagents for [carboxyl- ^{11}C] DL-tryptophan: 3-indoleacetaldehyde bisulfite addition compound, 0.1 mmole; $(\text{NH}_4)_2\text{CO}_3$, 0.6 mmole; NH_4Cl , 0.1 mmole; and KCN, 0.1 mmole.

All further operations through chromatographic purification are carried out in a high-level hot cell using manipulators. The 1 ml of 0.005 N NaOH containing trapped [^{11}C] cyanide is drained from the gas absorption column into the reaction vessel. The column is rinsed with 0.5 ml of distilled water and the rinse also added to the reaction vessel. Opening and closing the vessel is assisted by transfer to a 3/4-in. hexagonal socket mounted to a lead brick. The closed reaction vessel is then heated in a modified hot plate assembly regulated by a variable transformer (Fig. 4). The heating device is an aluminum block affixed to a hot plate and has a hexagonal hole ~ 2 in. deep and only slightly larger than the reaction vessel (to afford good heat transfer between the heating device and the reaction vessel). The

temperature is monitored using a mercury thermometer placed in an oil-filled hole in the block.

The synthesis time and temperature vary according to the C-11-carboxyl-labeled amino acid which is to be made. For neutral, aliphatic amino acids, the reaction time is 10 min for each of the two steps (see Fig. 4), and the reaction temperature is 210-220°C. For [carboxyl-¹¹C] DL-tryptophan the reaction times are 5 and 8 min for the first and second steps, respectively, and the reaction temperature is 240°C for both steps. We recently found that this change in the reaction parameters minimizes thermal decomposition of the intermediate hydantoin and produces higher and more reliable yields of [carboxyl-¹¹C] DL-tryptophan (1). For other aromatic amino acids, i.e., [carboxyl-¹¹C] DL-phenylalanine and DL-phenylglycine, the reaction time is 10 min for each step, and the reaction temperature is 190°C.

After the first heating step, the reaction vessel is cooled in an ice bath for ~ 30 sec, opened, and 1 ml of 6.25 N NaOH is added. The second heating step then results in hydrolysis of the intermediate hydantoin to the amino acid. After cooling as before, the valve is opened and the reaction mixture is removed using a syringe and blunt 4-in., 18-gauge needle. The reaction vessel is rinsed twice with 1 ml of distilled water and the rinses added to the reaction mixture.

PURIFICATION OF C-11-CARBOXYL-LABELED AMINO ACIDS

C-11-Carboxyl-labeled amino acids are purified by rapid column chromatographic techniques (Fig. 4). Columns are fitted with female ball joints to permit increased column flow rates by pressurization with ~ 7 psi of compressed air. In order to monitor the elution of radioactivity and thus minimize the elution volumes, the columns are eluted through tubing wrapped around a shielded gamma probe.

The purification procedure for neutral aliphatic and aromatic amino acids is as follows: In most cases, with the exception of C-11-carboxyl-labeled DL-valine, DL-phenylalanine, and ACBC, the reaction mixture is quite dark and should be filtered. This is accomplished by transferring the reaction mixture to a vertically-mounted 20-ml syringe body attached to a Millipore-type holder containing a coarse filter disc. The reaction mixture then is filtered by insertion of the plunger and expulsion of the liquid through the filter. The highly basic mixture is loaded directly onto a 1.5 cm x 5 cm, AG 1-X2, 100-200 mesh anion-exchange bed (Bio-Rad Laboratories, Rockville Center, NY), which has previously been converted to the hydroxide form by washing with 1 N NaOH. The column is rinsed twice with 30 ml of distilled water to remove neutral and cationic impurities and then eluted with 1 N HCl. The acidic eluate is loaded onto a 1.0 cm x 15 cm, AG 50W-X2, 50-100 mesh cation-exchange bed (Bio-Rad Laboratories) in the hydrogen form (previously washed with water and 1 N HCl). The column is rinsed twice with 30 ml of distilled water to remove anionic impurities, and the amino acid is eluted with 0.2 N NaOH. When activity is observed on the gamma probe, the column is allowed to flow slowly without pressurization, in order to elute the amino acid in a narrow band (~ 15 ml). The C-11-carboxyl-labeled amino acid solution is collected in a sterile 25-ml bottle and the activity measured in an ion chamber.

For [carboxyl- ^{11}C] DL-tryptophan a 1.0 cm x 3 cm column of 80-100 mesh Porapak Q (Waters Associates, Inc., Milford, MA) replaces the anion-exchange resin in the first purification step. 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 cooled reaction mixture is filtered as above, acidified with 2 ml of 6 N HCl, and then loaded onto the column. The loaded column is washed twice with 30 ml of 0.1 N HCl and is then eluted with 50% aqueous ethanol. The eluate is acidified with 2 ml of 6 N HCl (already present in the receiver) and loaded directly onto a cation-exchange bed as in the purification of neutral amino acids.

A total of approximately 40 min is required for synthesis and purification of C-11-carboxyl-labeled amino acids by our method. The solution is then transported to the Medical and Health Sciences Division of Oak Ridge Associated Universities for final radiopharmaceutical processing. This consists of neutralization to pH 7.0 ± 0.2 , filtration through a sterile 0.22 μ microfilter (Millipore Corporation, Bedford, MA) into a precapped, presterilized bottle, and a rapid (15 min) check for pyrogenic contamination using the Pyrotest Limulus amebocyte lysate technique (Difco Laboratories, Detroit, MI). The solution then is suitable for clinical use.

HIGH-LEVEL PRODUCTION OF C-11-CARBOXYL-LABELED AMINO ACIDS

Table 1 lists the amino acids which have been synthesized by our rapid, high-temperature, high-pressure Bücherer-Strecker technique. Most have been labeled with C-11, but a few were only labeled with C-14 using analogous procedures, because C-14 tissue distribution studies indicated that the C-11-labeled agents would not have significant nuclear medical potential. The C-11-carboxyl-labeled amino acids which are being investigated clinically (see Introduction) have all been produced many times; however, some of the other compounds have been made only once or a few times and the yield data may not be as indicative of the achievable yields. The chemical yields are based on the amount of stable cyanide used in the syntheses. The specific activity of C-11-carboxyl-labeled amino acids produced by this method generally is 15-40 mCi/mg at the completion of synthesis and purification. The low chemical yield for DL- α -methylvaline is probably due to steric hindrance.

In order to produce multiple batches of C-11-carboxyl-labeled amino acids in a given day, it is necessary to regenerate the chromatographic columns between batches. The anion exchange resin is regenerated by washing the column twice with 20 ml of water followed by washing with 40 ml of 3 N NaOH, and regeneration of the cation-exchange resin is analogous except that 40 ml of 3 N HCl is used in place of the NaOH. Regeneration of the Porapak Q is accomplished by rinsing the column with 20 ml of acetone, twice with 20-ml of water, and once with 20 ml of 0.1 N HCl.

Figure 5 shows how the various steps (cyclotron production of C-11 oxides, catalytic conversion and trapping of H^{11}CN , amino acid synthesis, amino acid purification, and column regeneration) are overlapped in order to produce multiple batches of various C-11-carboxyl-labeled amino acids at intervals of one hr. Further overlapping of the steps can result in production intervals as low as 45 min, but requirements regarding clinical turnover time

led to our routine production interval of approximately one hr. Over 120 batches of C-11-carboxyl-labeled amino acids have been produced for clinical tumor and pancreatic imaging studies over a three-year period.

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FIGURE LEGENDS

Figure 1.

Cutaway view of the B_2O_3 target assembly used for production of C-11 at the Oak Ridge National Laboratory's 86-Inch Cyclotron.

Figure 2.

Schematic of the system used for production of $^{11}CO/^{11}CO_2$, sequential catalytic conversion of $^{11}CO/^{11}CO_2$ to $H^{11}CN$, and trapping of $H^{11}CN$.

Figure 3.

Stainless steel reaction vessel and needle valve used for high-temperature, high-pressure modification of the Bücherer-Strecker amino acid synthesis.

Figure 4.

Schematic of the method used for production of C-11-carboxyl-labeled amino acids by our high-temperature, high-pressure modification of the Bücherer-Strecker amino acid synthesis.

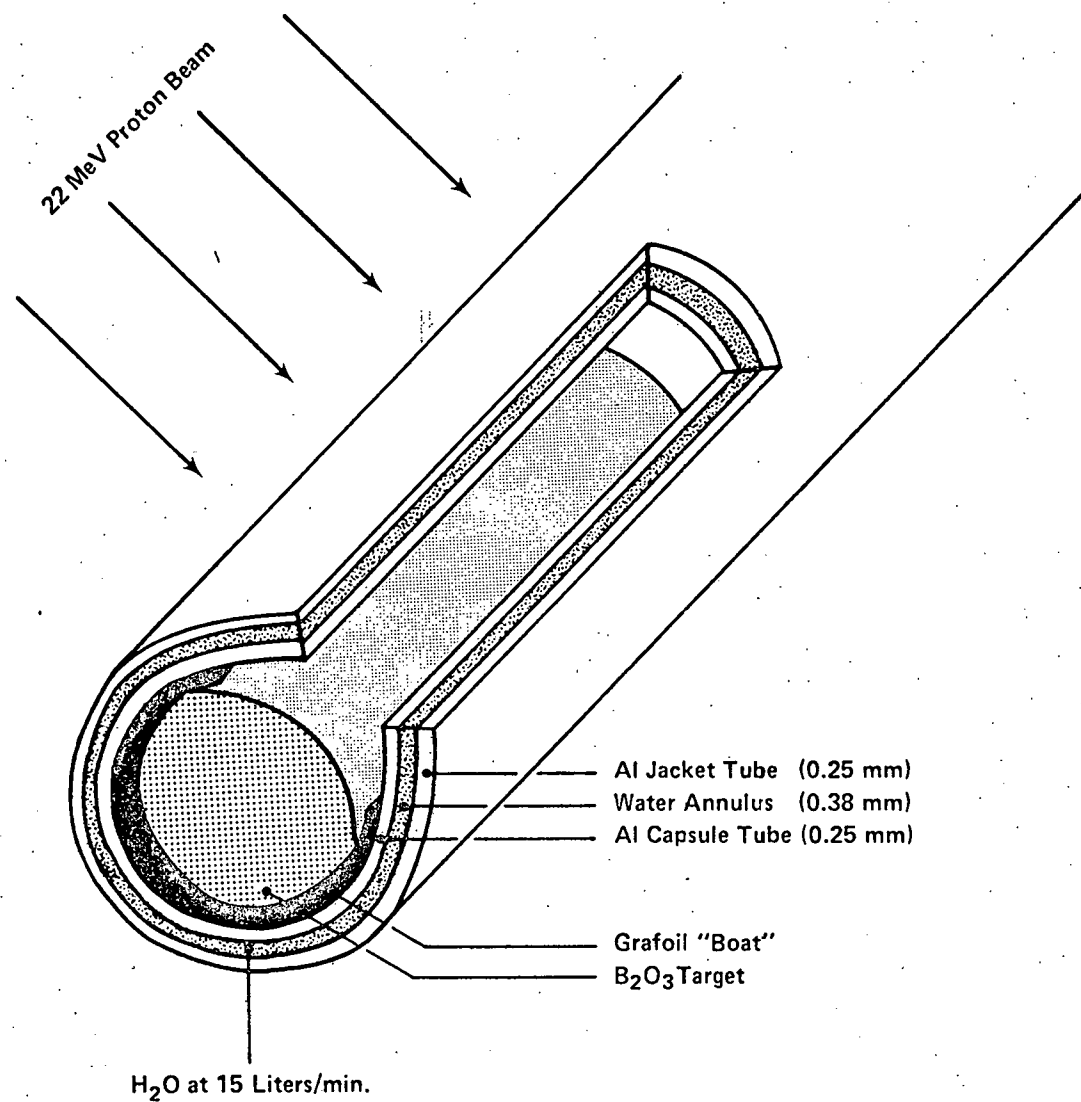
Figure 5.

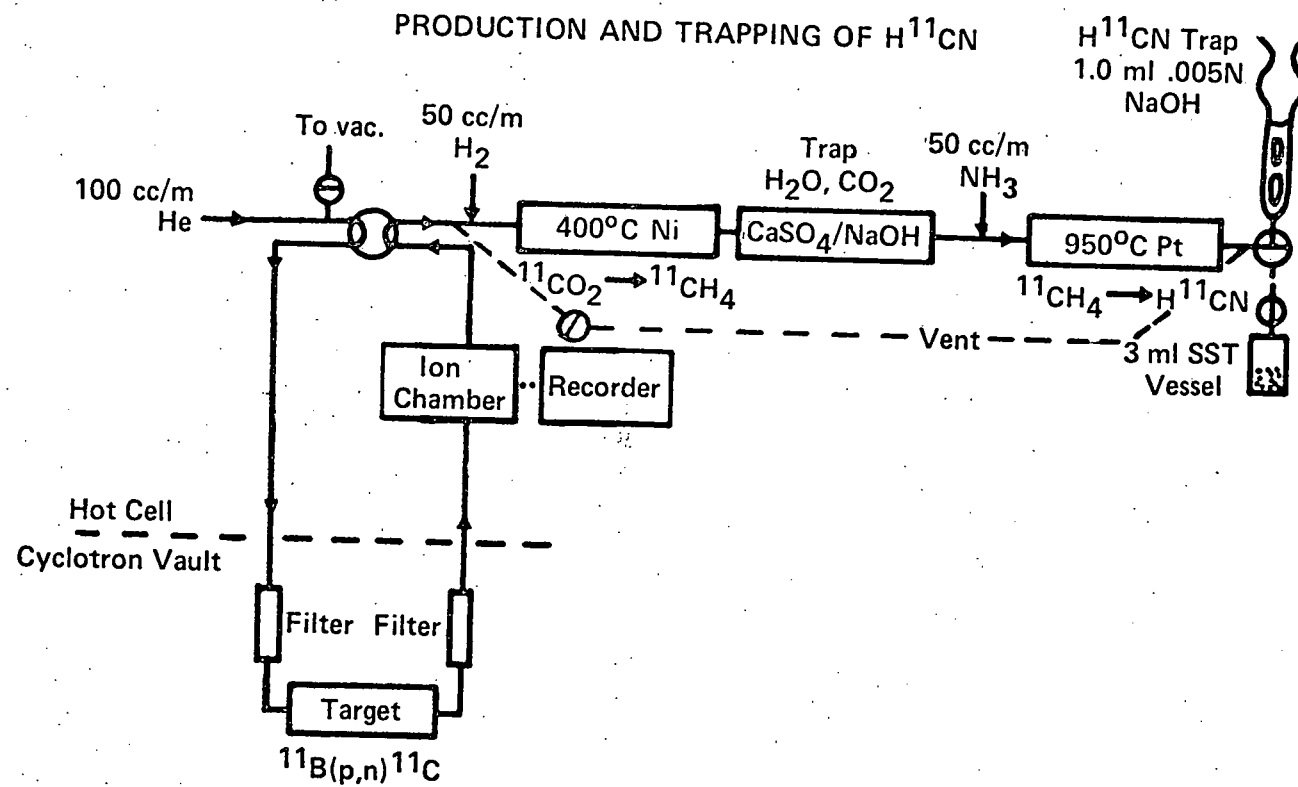
Timing of production steps for multiple batches of C-11-carboxyl-labeled amino acids at one-hour intervals.

TABLE 1. PRODUCTION OF VARIOUS PURIFIED AMINO ACIDS USING A HIGH-TEMPERATURE, HIGH-PRESSURE MODIFICATION OF THE
BÜCHERER-STRECKER TECHNIQUE

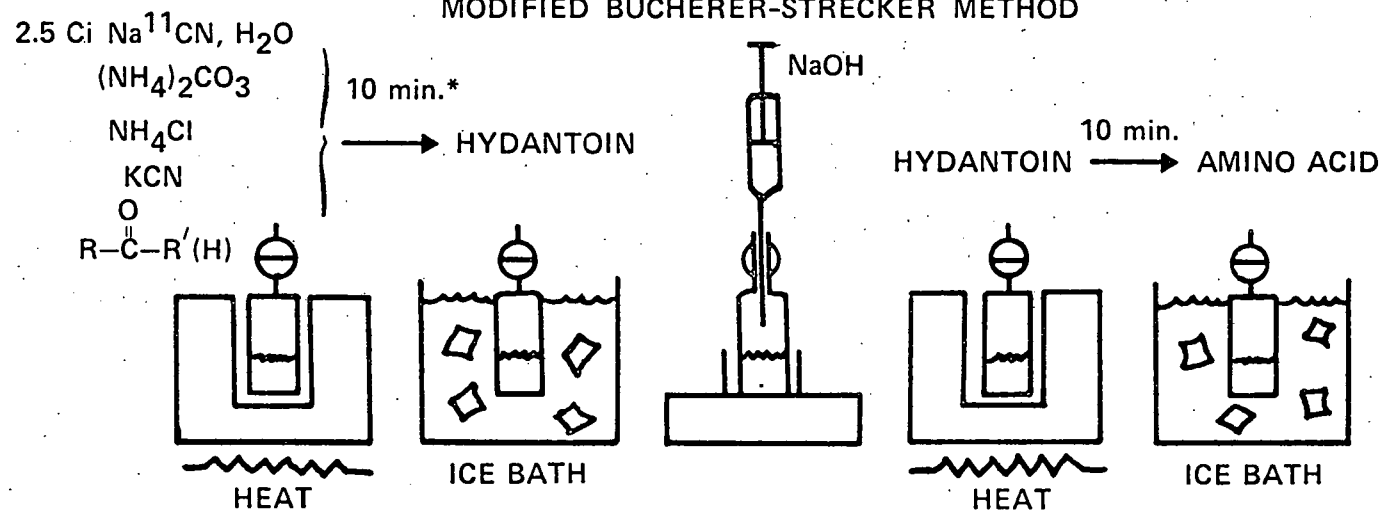
Amino acid	Precursor carbonyl compound	Batches C-11-labeled compound to date	Maximum chemical yield (%)	Average chemical yield* (%)	Maximum C-11 yield to date (mCi)
2-Aminobicyclo[2,2,1]heptane-2-carboxylic acid	Norcamphor	-	30	-	-
1-Aminocyclobutane carboxylic acid (ACBC)	Cyclobutanone	23	78	50	415
1-Aminocyclohexane-carboxylic acid	Cyclohexanone	1	55	-	75
1-Aminocyclopentane-carboxylic acid (ACPC)	Cyclopentanone	73	60	38	300
1-Amino-2-methylcyclopentanecarboxylic acid	2-Methylcyclopentanone	-	45	-	-
1-Amino-3-methylcyclopentanecarboxylic acid	3-Methylcyclopentanone	-	48	-	-
DL-Leucine	Isovaleraldehyde	-	45	-	-
DL- α -Methylvaline	3-Methyl-2-butanone	-	8	-	-
DL-Phenylalanine	Phenylacetaldehyde	4	68	55	425
DL-Phenylglycine	Benzaldehyde	1	31	-	150
DL-Tryptophan	3-Indoleacetaldehyde bisulfite addition compound	40	59	32	325
DL-Valine	Isobutyraldehyde	34	89	55	365

* Only for those amino acids which were synthesized four or more times.

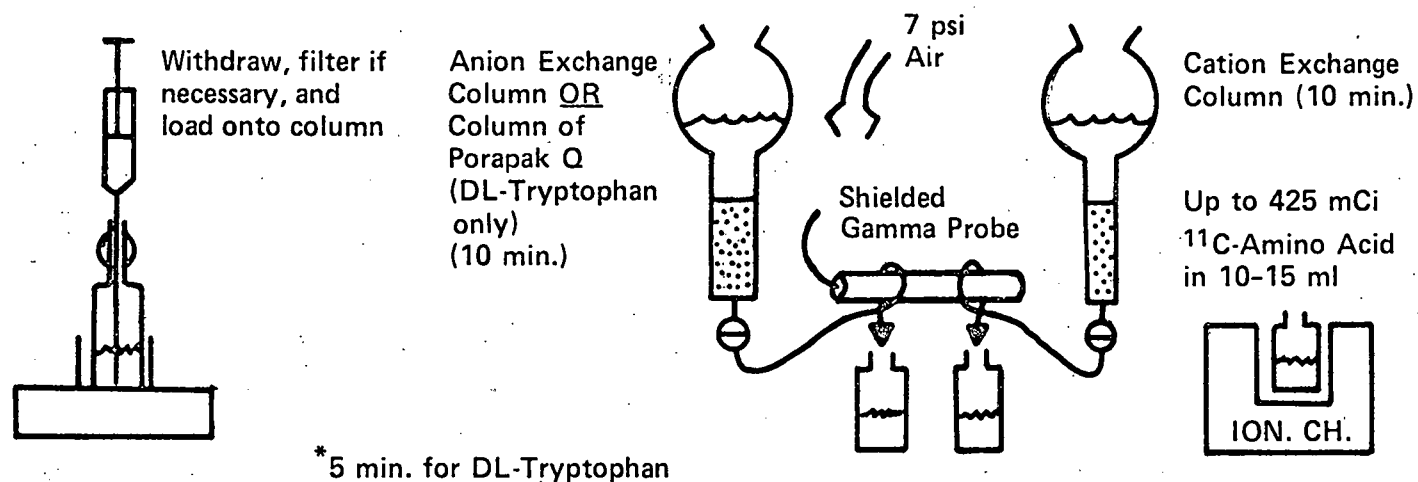




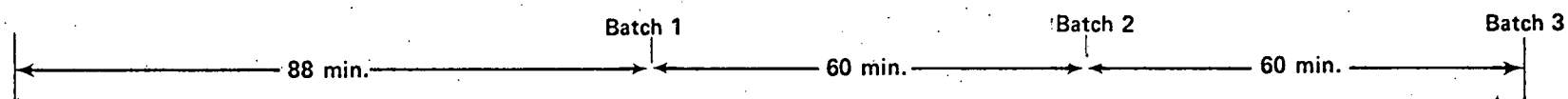
**^{11}C -AMINO ACID SYNTHESIS BY HIGH-TEMPERATURE, HIGH-PRESSURE,
MODIFIED BÜCHERER-STRECKER METHOD**



^{11}C -AMINO ACID CHROMATOGRAPHIC PURIFICATION



CONTINUOUS LARGE-SCALE PRODUCTION OF ANIMO ACIDS LABELED WITH CARBON-11



PRODUCE ¹¹ C OXIDES	H ¹¹ C N	SYNTHESIZE AMINO ACIDS	PURIFY AMINO ACIDS	REGEN. COLS.
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PRODUCE ¹¹ C OXIDES	H ¹¹ C N	SYNTHESIZE AMINO ACID	PURIFY AMINO ACID	REGEN. COLS.
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PRODUCE ¹¹ C OXIDES	H ¹¹ C N	SYNTHESIZE AMINO ACID	PURIFY AMINO ACID
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Step	Min.
Produce ¹¹ C	40
H ¹¹ CN	8
Synthesize	20
Purify	20
Regenerate	10