TECHNICAL NOTES

The Production in High Yield of N'-(4-[^{11}C]Methyl) -Imipramine

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A method for the routine production in high yield of N'-(4-[^{11}C]methyl)-imipramine is presented. The label is incorporated by reaction of C-11 methyl iodide ($^{11}CH_3I$) upon desipramine in dimethylsulfoxide. Quaternization of the tertiary amine by $^{11}CH_3I$ is minimized by using an excess of desipramine. The reaction proceeds at room temperature for 10 min and the product is isolated by means of high-performance liquid chromatography (HPLC). The entire production takes only 40 min and results in a radiochemical yield of 60%. About 60 mCi of labeled product are available for medical application; the specific activity, at the time of use, is 50 mCi/μmole. The product was characterized by chromatographic and spectrometric methods.

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Recent observations demonstrate that tritiated imipramine shows highly specific binding in the brain (*1*). It was suggested that the binding sites are related to the site of pharmacological action of some tricyclic antidepressants. Moreover H-3 imipramine might provide a biochemical marker for depression and the measurement of the density of these receptors could be a criterion for depression (*2*).

We labeled imipramine with the positron-emitting carbon isotope C-11 ($T_{1/2}$ = 20.4 min) for in vivo measurements using positron emission tomography. The radiopharmaceutical has to fulfill some conditions: the specific activity should be high enough to avoid saturation of specific receptors (H-3 imipramine used for in vitro tests (*2*) had a specific activity of 29.8 mCi per μmole); furthermore, since the starting material in the synthesis is desipramine—a tricyclic antidepressant that can displace imipramine from its binding sites—it is necessary to isolate C-11 imipramine from the initial reaction mixture with a highly specific procedure such as HPLC.

A method for the labeling of imipramine with C-11, based on the reductive methylation of desipramine using C-11 formaldehyde ($H^{11}CHO$), was reported by D. Comar et al. (*3*, *4*). Since in our hands the production of $H^{11}CHO$ by partial oxidation of $^{11}CH_3OH$, using a silver-wool catalyst at elevated temperature, did not always lead to reproducible results, we have developed a new method in which $^{11}CH_3I$ is used as precursor. Längström et al. (*5*) showed that nitrogen nucleophiles (e.g., secondary amines) can easily be alkylated with $^{11}CH_3I$ without dialkylation, on condition that the concentration ratio between the secondary amine and $^{11}CH_3I$ is large enough. C-11 methyl iodide is produced by our group in high yield on a routine basis.

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MATERIALS AND METHODS

Synthesis of C-11 methyl iodide. The production of $^{11}CH_3I$ is based on the method originally reported by Marazano et al. (*6*). Carbon-11 is formed by the ^{14}N (p,α)-μA beam intensity ^{11}C reaction (*7*) upon irradiation of nitrogen with 18-MeV protons (water-cooled, conical, aluminum target, internal diameter = 5–8 cm, length = 35 cm, N_2 pressure 10.5 bar) for 20 min at a 15-μA beam intensity. By releasing the pressure in the target and purging the system with a stream of N_2, $^{11}CO_2$ (formed by reaction of C-11 with trace amounts of O_2 present in the target) is led from the target into a flask containing 500 μmole of lithium aluminum hydride ($LiAlH_4$) in 0.5 ml of tetrahydrofuran (THF) at −80°C. By heating this mixture to 160°C the THF is evaporated, and $^{11}CH_3OH$ is released from the methanolate by adding 0.5 ml of 1*M* HCl. The labeled methanol is swept through hydroiodic acid at 180°C and the resulting $^{11}CH_3I$ is led through 0.5*M* NaOH, dried over P_2O_5, and trapped in the mixture for the C-11 imipramine synthesis.

Synthesis and HPLC of C-11 imipramine. Desipramine in the free base form is prepared from an alkaline aqueous solution of the hydrochloride salt by threefold extraction with diethyl ether. The organic phase is dried over anhydrous sodium sulfate and evaporated at 60°C, applying a gentle N_2 stream. Anhydrous dimethyl

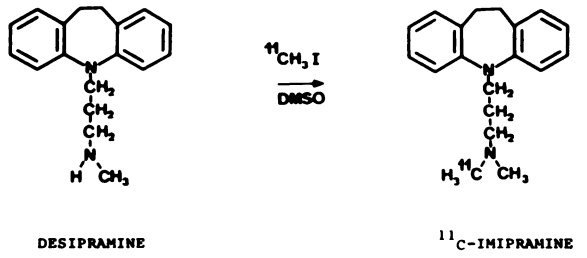

FIG. 1. Scheme for synthesis of C-11 Imipramine.

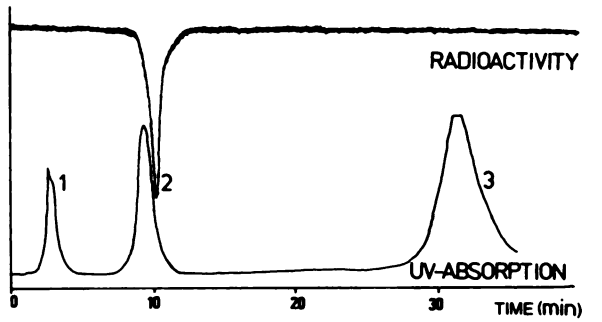

FIG. 2. HPLC chromatograms 1 = DMSO; 2 = C-11 Imipramine; 3 = Desipramine.

sulfoxide (DMSO) is added until a final concentration of 30 μmole of desipramine per 250 μl is obtained. The mixture is stored in a freezer, protected from light.

Two hundred and fifty microliters of this mixture are placed in a conical 5-ml vial. The $^{11}CH_3I$ is swept through the reaction mixture for 10 min via a hypodermic needle. The reaction, shown in Fig. 1, proceeds at room temperature. The reaction mixture is transferred through a peristaltic pump from the reaction vial to a second vial next to the injection loop of the HPLC apparatus*. In order to minimize loss of labeled product, 250 μl of isopropanol is used to flush the reaction vial and the tubing, and is added to the reaction mixture. This is then placed in the 500-μl injection loop and injected on the amino-bonded silica column [25 cm × 1 cm i.d.; Si-NH_2 particle size 10 μm (*8*)]. Before use the column is preconditioned with 250 ml of methanol containing 10% of isopropanol, followed by 250 ml of the eluent. The eluent consists of n-hexane/isopropanol/methanol/diethylamine, (98:2:0.2:0.05, v/v). The flow rate is 5 ml per min. Detection is performed by uv absorption at 254 nm, and activity by lead-shielded GM tube. Imipramine is retained for 9 min ($k' = 3.5$) and desipramine for about 30 min ($k' = 14$), showing the high selectivity of the Si-NH_2 column (separation factor $\alpha = 4.0$). Figure 2 shows a representative chromatogram. The radioactive imipramine fraction is led into a double-necked, conical, 100-ml vessel provided with a magnetic stirrer and placed in a boiling waterbath. Evaporation of the eluent is enhanced by applying a N_2 stream. The residue is taken up in 10 ml of a sterile isotonic saline solution containing 0.4% citric acid (w/v). Finally the solution is sent through a membrane filter (0.22 μm) and is collected in a syringe placed in a lead-shielded pot.

The whole production is remote-controlled and takes place in a shielded hot cell. Radioactivity is monitored by GM tubes at the different steps.

Identity and quality control. The HPLC method used for the isolation of the compound provides information concerning the chemical purity and the identity of the collected radioactive peak: a well-separated peak with a retention time equal to that of an imipramine standard.

Other identifications were carried out during the development of the method. For this purpose unlabeled methyl iodide was used under identical conditions. After HPLC, the fraction corresponding to imipramine was collected and the eluent removed by evaporation. The ir, uv, and mass spectra and the chromatographic behavior (HPLC, TLC, and GC) were identical with authentic material.

RESULTS AND DISCUSSION

Dimethyl sulfoxide, a highly polar aprotic solvent, was selected as a solvent since in this SN_2 reaction an increase of solvent polarity results in an increased reaction rate (*9*).

The method described permits the production of C-11 imipramine on a routine basis. The preparation, HPLC included, is completed by 40 min after the end of bombardment (EOB). The yield is 60 mCi of C-11 imipramine. Ninety percent of the $^{11}CH_3I$ produced is incorporated into the radiopharmaceutical, and the total radiochemical yield, based on the amount of $^{11}CO_2$ produced, is 60% at EOB. The amount of carrier is 1.2 μmole, resulting in a specific activity of 50 mCi/μmole. If necessary for medical applications, this figure can significantly be improved by a higher beam intensity and a longer irradiation time, and by using highly pure nitrogen gas in the target.

The product is obtained in a sterile, isotonic solution ready for medical use, and is chemically pure as shown by chromatography.

The procedure is simple and is easily incorporated into an on-line production system since it is a one-pot synthesis directly followed by HPLC. The use of remote-controlled apparatus also results in minimal radiation exposure to the personnel involved.

The method described has a higher yield than the one based on reductive methylation using H^{11}CHO. Indeed, Berger et al. (*4*) report a yield of 30% relative to CO_2.

The radiopharmaceutical has already been tested in rabbit experiments. These indicate that, shortly after administration, the radioactivity is already found in the brain and the lungs. As expected, high brain-to-blood ratios are found.

FOOTNOTES

* Waters Associates Chromatography pump equipped with a Knauer variable wavelength monitor and a linear recorder.

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