# Synthesis of [2-$^{11}$C]5,5-Dimethyl-2,4-oxazolidinedione for Studies with Positron Tomography

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**We have developed a method for the synthesis of [2-$^{11}$C]5,5-dimethyl-2,4-oxazolidinedione ([2-$^{11}$C]DMO) for use with positron emission tomography to measure regional tissue pH in vivo in man. [2-$^{11}$C]Dimethyl carbonate (DMC) was prepared from [C-11]phosgene and excess of sodium methoxide in methanol containing dimethyl carbonate as added carrier. The [2-$^{11}$C]DMC solution was then reacted with 2-hydroxyisobutyramide at 150 °C for 10 min to yield, after HPLC separation, [2-$^{11}$C]DMO with a radiochemical yield of 20–56%. Chemical yields were 78–92%, and specific activity ranged as high as 830 mCi/millimol.**

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An in vivo method for measuring brain tissue pH in humans would be useful in a number of clinical and experimental situations. Intracellular pH measurements in multicellular systems have been made by analyzing the tissue distribution of weak acids and bases and by means of pH-sensitive microelectrodes with tips small enough to avoid damage to the cell during penetration (*1–3*). In applying the weak-acid-distribution method, investigators have used $CO_2$ to measure cellular pH (*1*), and in particular intracellular pH in the brain (*4*). More recently, others have used $^{11}CO_2$ in combination with positron emission tomography (PET) to study cerebral acid-base chemistry in vivo (*5*). However, the technique of using $CO_2$ in intracellular pH measurements has been questioned seriously by others (*6*). On the other hand, 5,5-dimethyl-2,4-oxazolidinedione (DMO) appears to be the preferred weak acid for use with the weak-acid-distribution method (*2,7*) for the following reasons: (a) it has been validated by comparing it with the microelectrode method; (b) it is an active metabolite of the anticonvulsant trimethadione, a drug widely used in epilepsy, so its safety for humans has been well established (*8*); and (c) it does not metabolize to any significant extent (*9*). These considerations suggested to us that C-11-labeled DMO in combination with PET would be well suited for in vivo measurements of regional tissue pH in man.

Using twice Budinger's estimate of the root mean square deviation for PET (*10*), $2 \times 10^6$ counts will be required in a 10-cm-diam, 1-cm-thick brain slice to achieve 4.5% statistics in a 1-cm$^2$ region of interest. For a 15-min scan with our positron emission tomograph* (average slice sensitivity 30,000 cps/$\mu$Ci-cc), 0.1 $\mu$Ci/cc will be needed to obtain $2 \times 10^6$ counts. If we assume an uptake of 0.14% of the injected dose of DMO per 100 cc of brain and a 60-min preimaging equilibration period (L. Junck, personal communication), 50 mCi of [C-11]DMO will be required. Thus, 25 mg of 2 mCi/mg (250 mCi/millimol) would have to be injected intravenously into a man in order to obtain satisfactory images of the brain. As in the labeling of other pharmaceuticals with short-lived radionuclides, short reaction times and rapid separation techniques are essential in the synthesis of [C-11]DMO. With these considerations in mind, we examined a number of methods for labeling DMO in the 2 position using as the initial reactant [C-11]phosgene prepared essentially by the method of Roeda et al. (*11*). Although we have not explored all of the alternative schemes considered suitable for the synthesis of [C-

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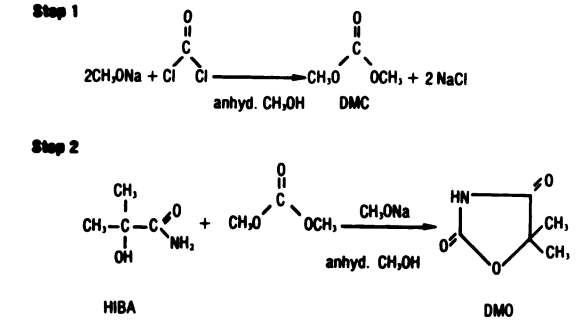


**FIG. 1.** Scheme for the synthesis of DMO.

11]DMO, we believe that the following scheme (Fig. 1) studied experimentally in some detail—first as a synthesis without labeled precursors and then in the incorporation of carbon-11—fulfills the aforementioned conditions. The preparation of 5,5-dialkyl-2,4-oxazolidinediones by reacting 2-hydroxyamide derivatives with diethylcarbonate or with a higher homolog in the presence of a metal alcoholate has been reported previously (*12*). Because of the relatively low specific-activity requirements, dimethyl carbonate (DMC), one of the reactants (Fig. 1, step 1), was used as a carrier to ensure high radiochemical yields.

## MATERIAL AND METHODS

**Synthesis of [C-11]phosgene.** The method of production was based on that described by Roeda et al. (*11*). The apparatus shown schematically in Fig. 2 was constructed entirely of stainless steel or monel tubing of various diameters joined together with Swagelok connectors. Nitrogen gas at a pressure of 45 psig was irradiated with 14.5-MeV protons in an aluminum target chamber (5 cm i.d., 100 cm long) to generate $^{11}CO_2$. A flow controller† F was used to reduce the pressure and control the rate of gas flow. A separate shut-off valve $V_1$ was used. The $^{11}CO_2$ was collected over a period of 8–10 min in a coil of monel tubing (⅛ in. o.d., 30 cm long) immersed in liquid nitrogen in a Dewar flask. A $P_2O_5$ trap (¼ in. o.d., 4 cm long) was used to remove water vapor. A three-way solenoid valve was used to direct the gas through a flowmeter at 500 cc/min during the collection of $^{11}CO_2$. The accumulated radioactivity was monitored with a 30-cc ion chamber connected to an electrometer.‡ When about 350 mCi of radioactivity (converted empirically from mR/min readings), had been collected, the gas from the target chamber was shut off and helium gas was used to purge the system and to act as carrier gas for the remainder of the operations. The helium flow rate was controlled by metering valve $M_1$. The solenoid valve $S_1$ was activated, causing the helium to pass through the zinc furnace, sodalime, and molecular sieve. The Dewar flask containing liquid nitrogen was lowered, and the $^{11}CO_2$ was allowed to evaporate and be carried through zinc powder§ contained in a monel tube (¼ in. o.d., 15 cm long) heated to 400 °C in a tube furnace, with monitoring by a furnace controller.‖ The $^{11}CO_2$ was reduced to $^{11}CO$ by the hot zinc and was passed through a sodalime trap (¼ in. o.d., 9 cm long) to adsorb any unconverted $^{11}CO_2$. The $^{11}CO$ was trapped over a period of 5–8 min in a molecular sieve 5A¶ contained in a U-shaped tube (⅛ in., 26 cm long) and cooled in liquid nitrogen. The flow rate through the molecular sieve was measured by a rotometer (0–50 cc/min). The radioactivity on the molecular sieve was monitored by a second 30-cc ion chamber connected to the single electrometer through a switch. The molecular sieve was allowed to warm to room temperature and was then heated to 150 °C in a tube furnace. When the radioactivity started to elute from the molecular-sieve column, a tube (⅛ in. o.d., 7.5 cm long) containing 0.45 g of $PtCl_4$ was inserted into a tube furnace preheated to 400 °C. The tube temperature, as measured with an attached chromel-alumel thermocouple, rapidly rose to 385 °C and thereafter increased gradually (10 °C/min). A tube (¼ in. o.d., 7 cm long) containing antimony powder was used to adsorb any unreacted $Cl_2$ evolved from the $PtCl_4$ (*11*). The [C-11]phosgene was bubbled through the reaction mixture of $NaOCH_3$ in methanol in a 1-cc conically shaped vial with septum cap. The time interval was 2–4 min between the release of $^{11}CO$ from the molecular sieve and the attainment of maximum activity in the reaction vial with the methanol solution of $NaOCH_3$.

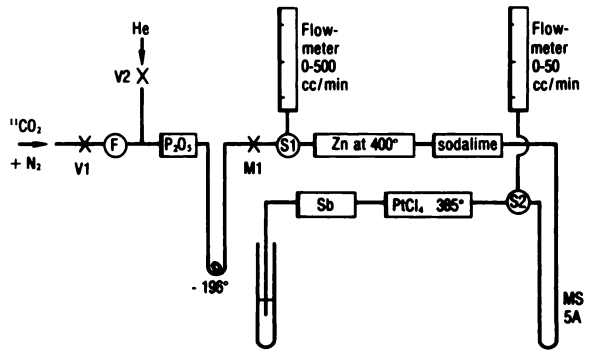


**FIG. 2.** Diagram of the [C-11]phosgene apparatus. $V_1$, $V_2$: shut-off valves; $M_1$: metering valve; $S_1$, $S_2$: three-way solenoid valves; F: flow controller.

**Synthesis of HIBA.** The precursor HIBA (Fig. 1, step 2) used in our synthetic scheme was prepared by allowing the methyl ester of 2-hydroxyisobutyric acid to react under anhydrous conditions with ammonia in an ammonia-saturated solution of methanol. This was prepared by bubbling anhydrous ammonia into a methanol solution of the ester cooled in ice water. The ice-water bath was removed and the temperature was allowed to rise to room temperature. After 20–24 hr, the same process was repeated and the reaction allowed to proceed for another 20–24 hr, after which the excess ammonia and methanol were removed under reduced pressure. The white solid residue was recrystallized from acetone-petroleum ether

to yield a crystalline product (85% of theoretical) with an mp of 92.5°-93.5° uncor. [lit. 93-94 °C (*13*)]. The identity and purity of the compound were further confirmed by TLC, HPLC, IR, and $^1$H NMR spectra.

**Synthesis of DMO.** Phosgene was allowed to react under anhydrous conditions with an excess of $NaOCH_3$ in methanol solution at ice-water temperature to form DMC (Fig. 1, step 1) rapidly and quantitatively. Without isolating the DMC, the solution was then used immediately in step 2 (Fig. 1), in which the reaction was carried out with near-stochiometric amounts of HIBA in a high-pressure steel vessel at approximately 150 °C for 10 min. Volatiles were removed under reduced pressure. The residue was dissolved in water, acidified with 6 *N* HCl to a pH of 2–3, and extracted exhaustively with ether, from which DMO was isolated in yields of 75–90% of theory based on HIBA. $^1$H NMR analysis of the residue showed it to contain only two organic substances: DMO and HIBA. The products were identified and characterized by mp, TLC, GC, HPLC, and $^1$H NMR spectra, and were compared with commercially available DMC and DMO.**

**Synthesis of [2-$^{11}$C]DMO.** A solution of 18.7 mg (0.208 millimol) of DMC in 426 $\mu$l of anhydrous methanol and an 85.6-$\mu$l methanol solution of $NaOCH_3$** containing 20.2 mg (0.374 millimol) of $NaOCH_3$, were transferred under anhydrous conditions with the aid of a Hamilton syringe into a 1 ml conically shaped mini-reaction vessel sealed with a septum cap. The [C-11]-phosgene was bubbled through the $NaOCH_3$–methanol solution and cooled in an ice-water bath to minimize losses of reagents as the helium carrier gas swept through. The [C-11]phosgene reacted rapidly to produce [2-$^{11}$C]DMC, the radioactivity of which was monitored with one of the 30-cc ion chambers. When the radioactivity reached a maximum, the contents were transferred to a high-pressure reaction vessel containing 18.4 mg (0.178 millimol) of HIBA, and the vessel was immediately placed in an aluminum block preheated to 150 °C while resting on a heating plate. After 10 min the reaction vessel was plunged into ice water to cool and the contents were then transferred to a 25-ml, pear-shaped flask and acidified to a pH 2.0–3.0 with two drops of 6 *N* HCl before volatiles were removed under high vacuum. The solid residue was dissolved and diluted with methanol–water (60:40) in a 10-ml volumetric flask, and checked for radiochemical purity by HPLC. Further dilution by a factor of 50 permitted us to determine yields by comparison with a reference solution of DMO of known concentration in a concentration range shown to be linear as a function of uv absorption. The specific activity of the [C-11]DMO was determined from the ratio of the radioactivity of the 10-ml solution of [C-11]DMO as measured in an ionization chamber†† with the number of millimoles of DMO contained in it as determined by HPLC analysis.

**Gas-chromatographic (GC) analysis.** For the identification and quantitative determination of DMC, a GC‡‡ equipped with a thermoconductivity detector and a Poropak Q column (1/4 in. × 6 ft) was used. Flow rate was 45 ml/min, injection and detector temperature 225 °C, and oven temperature 200 °C. Because DMC condensed in the cold tubing immediately after leaving the hot-wire detector (HWD), the radioactivity associated with it could not be determined by means of a flow radioactivity detector. Accordingly, short disposable glass pipettes were improvised with cotton at their tips, and these were inserted into the HWD gas outlet, close to the detector, for short constant time intervals corresponding approximately to the elution of methanol and DMC. The pipettes were then placed in a scintillation well detector and the radioactivity contained in them was measured. The background determined on a similarly improvised pipette but unexposed to GC gas effluents was subtracted.

**HPLC analysis.** This was performed on an instrument equipped with a variable uv detector set at 205 nm and with a flow radioactivity detector. A reverse-phase column§§ was used with methanol–water (60:40) as an eluant and a flow rate of 1.30 ml/min. An alternative eluant used was an aqueous phosphate buffer (pH 8.3, 6.6 m*M*) with a flow rate of 1.00 ml/min.

## RESULTS AND DISCUSSION

The entire synthesis and HPLC separation of [2-$^{11}$C]DMO required 28 min, and the chemical yield of DMO, based on the amount of HIBA, varied from 78 to 92%. The radiochemical yield based on the amount of $^{11}CO_2$ collected varied from 20 to 56%.

In those runs in which the $PtCl_4$ tube was kept in the furnace while raising the temperature to 385 °C, specific activities were low and varied from 22.7 to 32.2 mCi/millimol. By contrast, a total yield of as high as 137 mCi (sp. ac. 826.5 mCi/millimol) was obtained when the $PtCl_4$-containing tube was inserted into the furnace preheated to the prescribed temperature after $^{11}$CO began to elute from the molecular sieve at 150 °C. This underscores the importance of timing the heating of the $PtCl_4$-containing tube to the prescribed temperature (*14*) as the $^{11}$CO just begins to pass through it.

The chemical purity of DMC was confirmed by GC (methanol retention time = 53 sec, DMC retention time = 6 min 58 sec). Radioactivity was found in one peak corresponding to the retention time of [2-$^{11}$C]DMC. The radiochemical purity of [2-$^{11}$C]DMO was confirmed by HPLC. With methanol–water (60:40) as an eluant, the radioactivity was concentrated in one peak corresponding to the elution time of the reference DMO (1 min 43 sec). Under the same conditions, elution time for HIBA was 2 min 5 sec. With an aqueous phosphate buffer as an eluant and a flow rate of 1.0 ml/min, a better separation

was achieved between DMO and HIBA (DMO elution time = 1 min 54 sec, HIBA elution time = 3 min 56 sec). As much as 300 μg of DMO was separated from 276 μg of HIBA following a single injection (20 μl) using the same analytical column. One should be able to separate even higher amounts of DMO from HIBA by using a preparative column.

At present our efforts are aimed at preparing [2-$^{11}$C]DMO without added carrier. We hope to obtain a much higher specific activity, enabling us to prepare and purify by HPLC as much as 3–5 mg of [2-$^{11}$C]DMO.

## FOOTNOTES

* The Cyclotron Corporation, Model PC4600.

† Brooks Instrument Division, Emerson Electric Co., Model 8844B with a number 4 valve.

‡ Victoreen, Model 500.

§ Alpha Products, 100 mesh.

‖ Omega, Model 49-812.

¶ Supelco, 50 mesh.

** Aldrich Chemical Co.

†† Capintec, Inc., Radioisotope Calibrator CRC-10R.

‡‡ Perkin Elmer, Model Sigma 3B.

§§ Applied Science, Excalibar Spherisorb ODS m × 25 cm (SN 20346).

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## ERRATA

The article entitled "A Precision Flow-Controlled Rb-82 Generator for Bolus or Constant-Infusion Studies of the Heart and Brain" (*J Nucl Med* 22:1006–1010, 1981) was incorrectly classified as a Preliminary Note. It should have been identified as a Technical Note.

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In the article entitled "Simultaneous Determination of Free Thyroxine and Capacity of Thyroxine-Binding Globumin" (*J Nucl Med* 22:246–252, 1981), there are two typographical errors.

Equation (4) on page 248 is incorrect. The equation should read

$$(B/F) = \frac{\rho_s}{\rho_{T_4}}\alpha\left[V + \frac{\rho_s}{\rho_{T_4}}(I - V)\right]^{-1} - 1$$
$$= \frac{R_1}{R_0}\alpha\left[V + \frac{R_1}{R_0}(I - V)\right]^{-1} - 1. \quad (4)$$

On page 249, the column heading $FT_4$ should have the units ng/dl instead of μg/dl.