ROUTINE PRODUCTION OF 2-DEOXY-D-[1-^{11}C]GLUCOSE : AN ALTERNATIVE

D. Van Haver[a], N.A. Rabi[a], M. Vandewalle[*,a], P. Goethals[b] and C. Vandecasteele[+,b]

[a] Laboratory for Organic Synthesis, Krijgslaan, 281 (S.4)
[b] Institute of Nuclear Sciences, Proeftuinstraat, 86
Rijksuniversiteit Gent, B-9000 Gent (Belgium)

SUMMARY

The routine production of 2-deoxy-D-[1-^{11}C]glucose (^{11}C-2DG) from [^{11}C]hydrogen cyanide and 2,3:4,5-di-O-isopropylidene-1-iodo-D-arabinitol is described. The nitrile intermediate is purified by high-performance liquid chromatography (HPLC) before reduction/hydrolysis with Raney alloy in formic acid. The radiopharmaceutical is isolated by ion exchange and is obtained in a radiochemical yield of 20 % with a synthesis time of 50-55 min. A typical run affords 25-30 mCi of ^{11}C-2DG with a specific activity of 90-100 mCi/mg.

Key words : 2-deoxy-D-[1-^{11}C]glucose, [^{11}C]hydrogen cyanide, preparative HPLC, positron emission tomography.

INTRODUCTION

Positron emission tomography (PET) with 2-deoxy-D-[1-^{11}C]glucose (3, Fig. 1), or its 2-^{18}F analog, has evolved into an important technique for the study of regional cerebral glucose metabolism (1-3). An important advantage of ^{11}C over ^{18}F as the label is the possibility to administer the radiopharmaceutical at shorter time intervals in serial studies (4). Constraints on the synthesis time, due to the shorter half-live of ^{11}C (20.4 min), are compensated by the high available activities of [^{11}C]hydrogen cyanide, used as the precursor. Nevertheless, for the production of ^{11}C-2DG on a routine basis a reliable procedure remains necessary.

* Author to whom correspondence should be addressed.
+ Research Associate of the National Fund for Scientific Research (NFWO).

0362-4803/85/070657-10$01.00

Received September 25, 1984

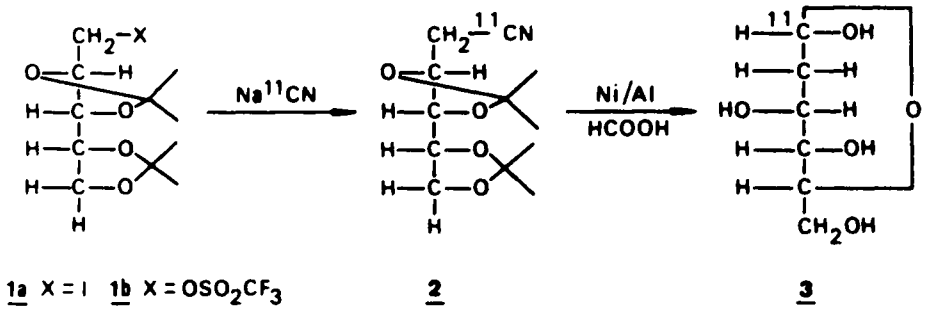

FIG. 1. Synthetic route to 2-deoxy-D-[1-^{11}C]glucose.

A synthesis of ^{11}C-2DG was first described by MacGregor et al. (4) and subsequently by Padgett et al. (5) and Vora et al. (6). The approach, as the one of Mestelan et al. (7) for ^{14}C-2DG, centers around the introduction of ^{11}C on a 2,3:4,5-di-O-isopropylidene-D-arabinitol-derived substrate 1 upon nucleophilic substitution with [^{11}C]cyanide ion, giving the corresponding [^{11}C]nitrile 2. Partial reduction of the nitrile function to the imine, subsequent hydrolysis to the aldehyde and removal of the isopropylidene protective groups then yields ^{11}C-2DG (3). Reduction/hydrolysis of the nitrile 2 with Raney alloy (Ni/Al) in formic acid, as applied by MacGregor et al. (4), is a convenient method for the critical conversion to the aldehyde. Strict stoechiometric requirements don't have to be met, no anhydrous conditions are needed and the isopropylidene groups are cleaved simultaneously. It is, however, not suited for the conversion of crude 2 obtained from the iodide 1a, as the byproducts poison the metal alloy used for the reduction (4). The starting material now commonly used is the triflate 1b (4-6), a reactive substrate for displacement by the cyanide group, but being sensitive to hydrolysis this precursor needs to be prepared shortly before use.

We present an alternative for the routine production of ^{11}C-2DG, allowing the intermediacy of the iodide 1a upon including a purification step of the [^{11}C]nitrile 2 prior to the reduction/hydrolysis with Raney alloy in 25 % formic acid. Unlike the triflate 1b, the iodide 1a is stable ; it can be prepared on a large scale and can be stored almost indefenitily (7). In addition, the puri-

fication procedure for the ^{11}C-2DG radiopharmaceutical has been modified to include only ion-exchange chromatography. A further simplification is achieved by avoiding evaporation steps for removal of water or high-boiling solvents at any time during the production.

MATERIALS AND METHODS

2,3:4,5-Di-O-isopropylidene-1-iodo-D-arabinitol (1a) was prepared from D-arabinitol (Fluka AG) according to Mestelan et al. (7). It was purified on a 10-µm silica HPLC column (50 x 0.68 cm) with ethyl acetate-n.hexane 6:4 (v/v) as eluent and stored as a 0.05 M solution in dry N,N-dimethylformamide. [^{11}C]Hydrogen cyanide is produced by a modification, described in detail in reference 8, of the method of Christman et al. (9).

The synthesis system for ^{11}C-2DG is shown in Fig. 2. The parts of the system are interconnected by lengths of Teflon tubing (1.6 mm OD) either through inert fittings or, terminated with a hypodermic needle, inserted through the septum stoppers used for sealing the reaction and storage flasks. The necessary vents are made in the same way. Syringes on external access tubing are used to add reagents and/or solvents from outside the hot-cell. The reaction flasks C and G are each mounted on an electrically driven plunger (Stafa Control System AlH30) to move them in and out a Wood's-metal heating block. The HPLC sample injection valve D (Valco CV-6-UHPa-N60) is fitted with a 0.5 ml sample loop and the shaft is lengthened through the wall of the hot-cell for remote operation. The ion-exchange resins (Bio-Rad) for the purification of ^{11}C-2DG are packed in glass columns terminated on both sides with a length of 6.4-mm OD tubing (filled with glass-wool) to fit the tube connectors (Swagelok). Four three-way solenoid valves (Nacom NC-C-NO) allow to select the supply to the columns K and L, and to collect the product-containing fraction from the columns F and L. All solutions are transferred by means of small peristaltic pumps (Ismatec Mini-S 840 and Pharmacia P-1); for the elution of the coupled columns K and L, giving a high back-pressure, a larger pump (Gilson Minipuls 2 HP2/HF; 3-mm ID flow tube) is used. The effluent from the columns F, K and L is monitored by a lead-shielded radioactivity detector (GM tube).

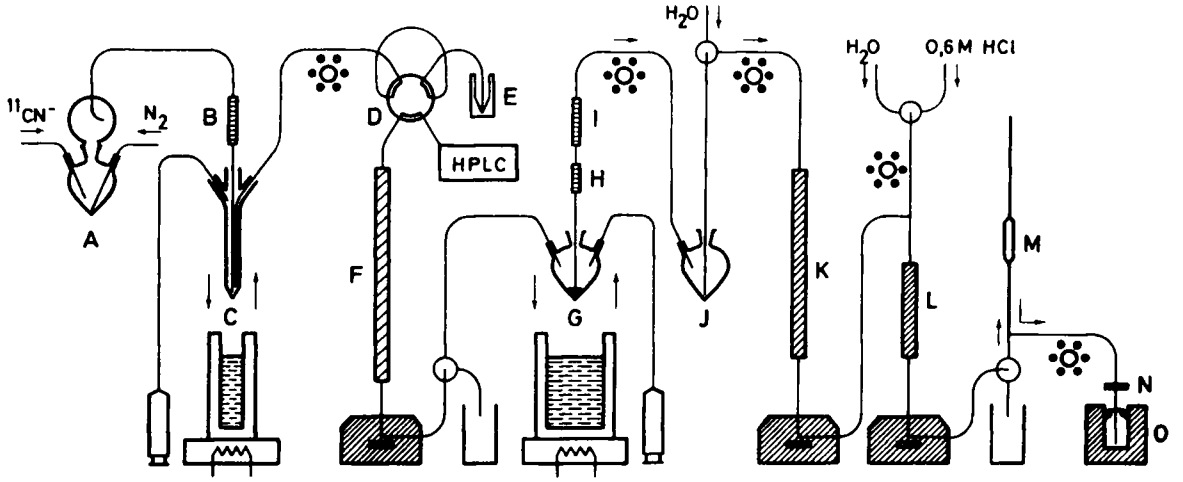

FIG. 2. Synthesis system for ^{11}C-2DG : A. H_2SO_4; B. P_2O_5 trap; C. substitution reaction; D. sample injection valve; E. dilution vial; F. reverse-phase column; G. reduction/hydrolysis; H. glass-wool filter; I. AG50W-X8/H^+ column; J. effluent collection; K. AG1-X2/PO_4^{3-} column; L. AG1-X2/OH^- column; M. 2-ml pipette; N. sterilization filter; O. product vial.

[^{11}C]Hydrogen cyanide (8) is trapped in a solution of 2 μmoles of sodium cyanide in 1 ml of water contained in a 5-ml pear-shaped flask (not shown in Fig. 2). The [^{11}C]cyanide solution, followed by 1 ml of water, is transferred to a 10-ml three-neck flask (A) containing 0.5 ml of 9 M sulfuric acid. Nitrogen gas is then bubbled through the solution for 5 min at a flow rate of 90 ml/min, sweeping the liberated [^{11}C]hydrogen cyanide through a phosphorus pentoxide trap (B, 3 x 0.7 cm) into a mixture of 0.1 ml of the stock solution of 1a (50 μmoles) in N,N-dimethylformamide and 0.1 ml of 0.05 M sodium hydroxide (5 μmoles)/0.07 M sodium cyanide (7 μmoles) in dimethylsulfoxide contained in a cylindroconical three-neck flask (C, 10 x 1.0 cm). At all times a nitrogen flow through one of the necks prevents moisture and carbon dioxide from entering into the solution. During the trapping of [^{11}C]hydrogen cyanide the solution is cooled to ca. -15°C by occasionally condensing dry ice on the wall of the flask.

The substitution reaction is effected by heating the solution at 100°C for 4 min. The flask is raised from the heating bath and the reaction mixture is transferred to a 2-ml vial (E) connected to the waste outlet of the HPLC sample injection valve (D). Flask C and the connecting tubing are then flushed with 0.3 ml of 0.1 M phosphate buffer pH 5.3. The diluted reaction mixture is pumped into the sample loop and injected on the HPLC column (F)(10).

The [^{11}C]nitrile 2 is purified on a 10-μm RSiL-C18HL reverse-phase column (25 x 0.46 cm, Alltech Europe) with water-n.propanol 3:1 (v/v) as eluent; at a flow rate of 1.0 ml/min it is eluted after 7 min. A typical chromatogram is shown in Fig. 3. Upon detection of ^{11}C activity from 2 in the column effluent, a 1.5-ml fraction is collected in a 5-ml pear-shaped flask (G) equipped with a magnetic stir bar and containing 150 mg of Raney alloy (Ni/Al 1:1); 0.5 ml of formic acid (98 %) is added simultaneously from outside the hot-cell. Conversion of 2 to 3 is then achieved by heating the solution with stirring at 100°C for 10 min.

Through a hypodermic needle (3.0 mm ID) which reaches the bottom of the flask when it is raised from the bath, the reaction mixture is passed through a glass-wool filter (H) coupled with a AG50W-X8 (200-400 mesh) column (I, 4 x 1.0 cm) in the H^+ form.

Flask G and the filtered alloy are rinsed with 3 ml of distilled water which are also applied to column I. Elution is continued to dryness of the column and the eluate, collected in a 10-ml pear-shaped flask (J), is brought in the flow of distilled water to column K by operating the proper three-way valve. Filling the connecting tubing beforehand with distilled water and careful timing of valve switching avoids disturbing the operation of the peristaltic pump by air.

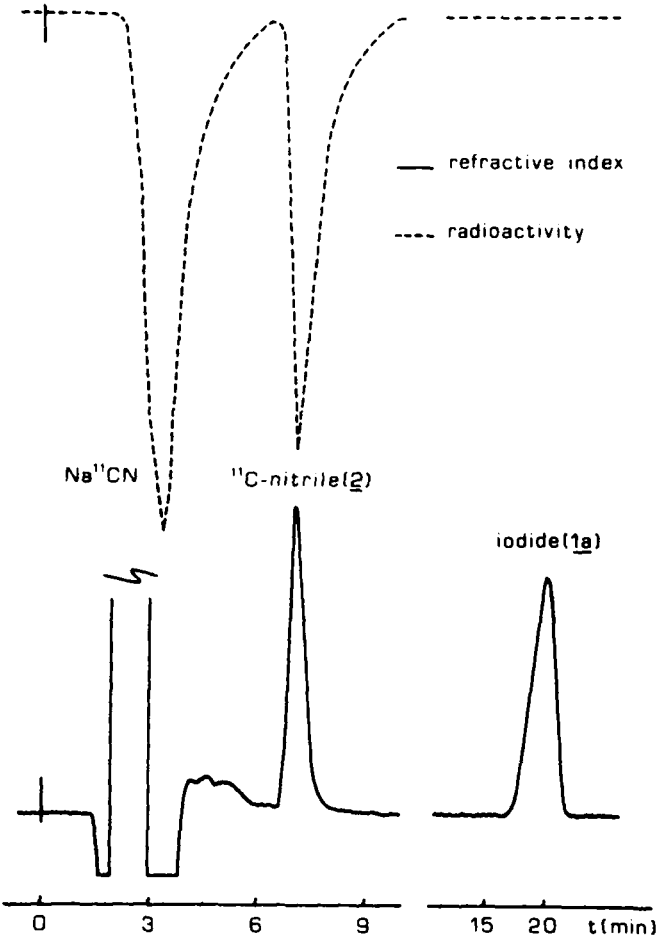

FIG. 3. Chromatogram of the preparative HPLC purification of the [^{11}C]nitrile 2. Conditions as described in the text.

The solution is applied to a AG1-X2 (100-200 mesh) column (K, 14 x 1.4 cm) in the PO_4^{3-} form, joined through a T-connector to a AG1-X2 (100-200 mesh) column (L, 3 x 1.0 cm) in the OH^- form, and is eluted with distilled water (flow rate : 7 ml/min). A gradual color change of the resin to yellow is observed when formic acid is adsorbed on column K. Upon completion of the elution of ^{11}C-2DG from the column, signaled by the disappearence of ^{11}C activity in the effluent, column L, now retaining ^{11}C-2DG, is rinsed further with distilled water (3 min; flow rate : 6 ml/min) to ensure that all non-acidic solutes are removed. The column supply is then switched to 0.6 M hydrogen chloride for eluting ^{11}C-2DG; a marked change in color accompanies the adsorption of hydrogen chloride. As soon as ^{11}C activity is detected, 2 ml of column effluent are collected in a sterilized pipette (M).

The solution is then passed through a 0.22-µm Millipore filter (N) and collected in a sterile vial (O) containing 1 ml of 0.6 M sodium hydroxide and 5 ml of 0.125 M phosphate buffer pH 7.0. At this point, a neutral and isotonic solution (8 ml) of ^{11}C-2DG is ready for injection.

The ^{11}C-2DG produced was identified by comparison with a sample of 2-deoxyglucose (Aldrich) using HPLC (25 x 0.46-cm RSiL-NH2 column, acetonitrile-water 72:28, flow rate 2 ml/min) and TLC (Merck Si-60, ethyl acetate-pyridine-water 7:3:2, 10 % sulfuric acid spray), and was found to be > 95 % pure. The radiochemical purity was > 98 %, as demonstrated by thin-layer radiochromatography. Tests showed the solution for injection to be sterile and pyrogen free.

RESULTS AND DISCUSSION

The initial aqueous [^{11}C]cyanide solution is treated with sulfuric acid to yield [^{11}C]hydrogen cyanide, since it contains the precursor as [^{11}C]ammonium cyanide, accompanied by a large amount (300-600 µmoles) of ammonia (8). Attempts to liberate [^{11}C]hydrogen cyanide from this solution by passage through a small cation-exchange column, or to obtain [^{11}C]sodium cyanide directly by evaporation of the solvent (95 % ethanol in this case) in the presence of sodium hydroxide, resulted in extensive losses. The sulfuric acid method allows to transfer 80 % of the initial ^{11}C acti-

vity into the starting solution for the nucleophilic substitution reaction, provided that this contains at least 5 µmoles of sodium hydroxide.

The yield of the substitution reaction under these conditions is 40 % and is obtained after 3-4 min. Prolonged heating caused no drop in yield, contrary to the observations of Mestelan et al. (7).

The main reason for the purification of 2 is the removal of unreacted iodide 1a. Indeed, the low yield obtained for ^{11}C-2DG upon reduction/hydrolysis of 2 in the presence of pure 1a suggests that the latter is the main cause of poisoning of the metal alloy. Dilution of the crude reaction mixture of 2 with buffer pH 5.3 before injection on the HPLC column serves three purposes : it rinses the reaction flask (C), it prevents the column packing from deteriorating by repeated injection of an alkaline solution and it allows the purification to be performed on an analytical column (25 x 0.46 cm), thus minimizing the elution volume of 2. The use of n.propanol (b.p. 97°C) in the eluent allows this to be used as solvent for the subsequent reduction, so that an additional evaporation step is avoided. The reproducibility of the separation is such that in routine production refractive index detection (Fig. 3) can be omitted.

The conversion of 2 into ^{11}C-2DG is only mildly affected by the presence of n.propanol in the reaction medium. The yield is 55 %, as compared to 60-70 % in water (4).

Removal of formic acid from the ^{11}C-2DG solution by passage through an anion-exchange resin in the PO_4^{3-} form is based on the "site sharing" principle (11). The penetrating formic acid (pK 3.8) forms $H_2PO_4^-$ counter ions through protonation, but it is too weak an acid to release undissociated phosphoric acid (pK_1 2.1; pK_2 7.2; pK_3 12.7) from the resin. The net effect is an uptake of formic acid without displacement of the original counter ions. 2-Deoxyglucose cannot donate protons even to PO_4^{3-} ions and passes unadsorbed through the column. This method is more convenient than neutralisation with an anion-exchange resin in the HCO_3^- form. In that case the resin is preferencially stirred in the acid solution

and filtered off (6) to cope with the evolution of carbon dioxide upon adsorption of formic acid. Packing the resin in a column (4) led in our hands to unacceptable losses of ^{11}C-2DG due to the disturbance of the resin bed.

As demonstrated by HPLC and TLC analysis, a strongly basic anion-exchange resin in the OH^- form allowed to concentrate 2-deoxyglucose from the effluent of column K and to purify it to an adequate level by preferential adsorption over the accompanying solutes. This simple purification procedure is possible because of the high purity of the starting [^{11}C]nitrile 2 which upon reduction/hydrolysis yields ^{11}C-2DG without formation of acidic by-products. Since carbonic acid has a higher selectivity for the exchanger than 2-deoxyglucose, it is important to convert the ion-exchange resins to the proper ionic form and to elute the columns during the production using distilled water carefully freed from carbon dioxide.

The production of [^{11}C]hydrogen cyanide in our experimental set-up requires an additional 6-7 min as compared to the known procedure (9), due to the treatment of the initial alkaline [^{11}C] cyanide solution with sulfuric acid. The synthesis time of ^{11}C-2DG from [^{11}C]sodium cyanide is 50-55 min (65-70 min from EOB), including the purification step of the [^{11}C]nitrile 2. This is comparable to the time needed for the procedures reported so far (4-6). The overall yield based on the ^{11}C activity trapped in the starting solution for the displacement reaction by cyanide is ca. 20 %. In a typical run (irradiation time : 30 min; beam intensity : 25 µA) 900-1000 mCi of [^{11}C]sodium cyanide are available for reaction 15 min after EOB. From this 25-30 mCi of ^{11}C-2DG are obtained 50-55 min later. As the total amount of sodium cyanide carrier added is 8-9 µmoles, 1.6-1.8 µmoles of ^{11}C-2DG are produced. The specific activity at the time the solution is ready for injection is therefore 90-100 mCi/mg.

The preference of the iodide 1a over the triflate 1b as starting material makes the presently described method more convenient for routine production. The modification presented for the purification of ^{11}C-2DG is short and simple to perform. Sixteen pro-

ductions for clinical studies have been performed, showing the reliability of the procedure and synthesis system for the ^{11}C-2DG radiopharmaceutical.

ACKNOWLEDGEMENTS

The authors thank Prof. J. Hoste for his interest in this work, E. Gillis and J. Sambre for technical assistance and the NFWO and IIKW for financial support. This work was supported by a grant of the Belgian government (Geconcerteerde Onderzoeksactie 80/85-6).

REFERENCES

1. Sokoloff L. - Brain 102: 653 (1979).
2. Fowler J.S., Lade R.E., MacGregor R.R., Shiue C.-Y., Wan C.-N. and Wolf A.P. - J. Lab. Comp. Radiopharm. 16: 7 (1979).
3. Phelps M.E. - Semin. Nucl. Med. 11: 32 (1981).
4. MacGregor R.R., Fowler J.S., Wolf A.P., Shiue C.-Y., Lade R.E. and Wan C.-N. - J. Nucl. Med. 22: 800 (1981).
5. Padgett H.C., Barrio J.R., MacDonald N.S. and Phelps M.E. - J. Nucl. Med. 23: 739 (1982).
6. Vora M.M., Boothe T.E., Finn R.D., Smith P.M. and Gilson A.J. - J. Lab. Comp. Radiopharm. 20: 417 (1983).
7. Mestelan G., Aubert F., Beaucourt J.-P., Comar D. and Pichat L. - J. Lab. Comp. Radiopharm. 16: 661 (1979).
8. Sambre J., Vandecasteele C., Goethals P., Rabi N.A., Van Haver D. and Slegers G. - Int. J. Appl. Radiat. Isotopes (in press).
9. Christmann D.R., Finn R.D., Karlstrom K.I. and Wolf A.P. - Int. J. Appl. Radiat. Isotopes 26: 435 (1975).
10. Vandewalle T., Vandecasteele C. and Van Haver D. - Radiochem. Radioanal. Letters 51: 29 (1982).
11. Helfferich F. - Ion Exchange, McGraw-Hill, New York, 1962, p. 148.