Appl. Radiat. Isot. Vol. 40, No. 1, pp. 57–61, 1989
Int. J. Radiat. Appl. Instrum. Part A
Printed in Great Britain.

0883-2889/89 $3.00 + 0.00

Synthesis of ^{11}C-Labelled Thymidine for Tumor Visualization Using Positron Emission Tomography

E. POUPEYE,[1] R. E. COUNSELL,[2] A. DE LEENHEER,[1]* G. SLEGERS[3] and P. GOETHALS[4]

[1]Laboratoria voor Medische Biochemie en voor Klinische Analyse, Rijksuniversiteit Gent, Harelbekestraat 72, B-9000 Gent, [2]Department of Pharmacology, M 6322 Medical Science Building 1, The University of Michigan, Medical School, Ann Arbor, MI 48109-0624, U.S.A., [3]Laboratorium voor Analytische Chemie and [4]Instituut voor Nucleaire Wetenschappen, Faculteit Farmaceutische Wetenschappen, Rijksuniversiteit Gent, Harelbekestraat 72, B-9000 Gent, Belgium

(*Received* 25 *February* 1988; *in revised form* 21 *April* 1988)

A no-carrier added synthesis of [^{11}C]thymidine from cyclotron produced [^{11}C]methyl iodide is presented. The bis-silylated derivative of the lithium salt of 5-bromo-2′-deoxyuridine is treated with [^{11}C]methyl iodide in THF at −50°C giving 20–30 mCi [^{11}C]thymidine in a 40–70% radiochemical yield. Purification is performed by reversed phase chromatography on a C_{18} column. The whole procedure takes 25 min and the specific activity is about 20–30 mCi/μmol at the end of the synthesis.

Introduction

There are several reasons to believe that [^{11}C]thymidine has considerable potential for tumor visualisation and liver regeneration studies by positron emission tomography (PET). Rapidly dividing cells are known to have high activities of thymidine kinase (Hampton *et al.*, 1982), an enzyme responsible for phosphorylating thymidine to produce thymidyllic acid. Since the latter compound cannot transverse the cellular membrane (Plunkett and Cohen, 1977) the thymidine remains trapped in the tumor cells. Moreover, studies with [methyl-^{14}C]thymidine and [methyl-^{3}H]thymidine indicate that tumor uptake and tumor/tissue ratios are sufficiently high to use [methyl-^{11}C]thymidine for the PET imaging (Crawford *et al.*, 1978; Sundoro-Wu *et al.*, 1984).

In 1972 [methyl-^{11}C]thymidine was prepared by biosynthesis using thymidylate synthetase for incorporation of [^{11}C]formaldehyde into the methyl group of thymidyllic acid followed by a dephosphorylation using *E. coli* alkaline phosphatase. This enzymatic synthesis only produced 4 mCi [^{11}C]thymidine with low specific activity in 110 min. Application of the [^{11}C]thymidine to human studies would require higher radioactivities and additional purification steps increasing the overall time required for the production of the radiopharmaceutical (Christman *et al.*, 1972; Crawford *et al.*, 1978).

Langström suggested a synthesis using [^{11}C]methyl iodide and the tris-(trimethylsilyl) derivative of the lithium salt of deoxyuridine. The reaction reportedly proceeded at −78°C in tetrahydrofuran–ether mixtures, containing sparteine as a metal complexing diamine. As recognized by the author himself, this method suffered from low yield, poor reproducibility and a long reaction time (40 min). The low reactivity of the tris-(trimethylsilyl) derivative of the lithium–deoxyuridine as well as uneffective hydrolysis of the silyl ether after the reaction (using water rather than acid) both presumably contributed to the poor overall yield. Furthermore, details on the purification method and the total radioactivity produced were not presented (Langström, 1980; Langström *et al.*, 1981).

In an alternative synthesis based on the former one, bis-(tetrahydropyranyl)-lithiumdeoxyuridine was treated with [^{11}C]methyl iodide to produce 2.4–4.7 mCi [^{11}C]thymidine in 7–25% radiochemical yield within 45 min. The low specific activity was due to the addition of carrier methyl iodide (Sundoro-Wu *et al.*, 1984).

The aim of our work was to eliminate the drawbacks of the existing methods for the synthesis of [^{11}C]thymidine, i.e. the low yield, low specific activities of the end product and the long reaction times. Therefore we adapted a procedure previously reported by Pichat for the preparation of [^{14}C]thymidine. His approach was based on the reaction of the lithium-bis-(trimethylsilyl)-5-bromo-2′-deoxyuridine with [^{14}C]methyl iodide at −60°C in tetrahydrofuran and hexamethylphosphorictriamide as cosolvent (Pichat *et al.*, 1971). The use of

*Author for correspondence.

this carcinogenic chemical as well as the complexity of the procedure (3 h reaction time and a lengthy purification scheme using several chromatographic steps) are obviously prohibitive for any work with short-lived isotopes. In this paper we report several substantial improvements to the original approach of Pichat resulting in a convenient high yield synthesis of [^{11}C]thymidine. Key-features of our method are the use of bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) for the preparation of the bis-(trimethylsilyl)-5-bromo-2′-deoxyuridine, the effective hydrolysis of silyl ethers in acid and the simplified purification procedure involving an extraction with chloroform and reversed phase chromatography (HPLC). The whole procedure takes about 25 min (10 min reaction and 15 min purification) and gives [^{11}C]thymidine in high yield with high specific activity.

Experimental

Solvents and reagents

5-Bromo-2′-deoxyuridine, bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), calcium hydride, n-butyl lithium, pyridine and tetrahydrofuran (THF) were purchased from Janssens Chimica (Beerse, Belgium). Pyridine and THF were freshly dried over calcium hydride and purified by distillation.

Thin layer chromatography was performed on precoated silicagel 60F_{254} aluminium sheets obtained from Merck (Darmstadt, F.R.G.). Solvents used for high performance liquid chromatography (HPLC) were high purity grade and were obtained from Merck.

Methods

Preparation of [^{11}C]methyliodide. [^{11}C]methyliodide was produced according to Denutte *et al.* (1982).

Preparation of 3′,5′-o-bis(trimethylsilyl)-5-bromo-2′-deoxyuridine (BSBDU). The hydroxyl groups of 5′-bromo-2′-deoxyuridine were protected by silylation (Fig. 1). The reaction was performed in a 5-mL flask with a septum inlet, flushed with dry N_2.

Dry pyridine (1 mL) was added to the flask containing 5 mg (16.3 μmol) dried 5-bromo-2′-deoxyuridine. After complete dissolution, 1 mL BSTFA was added. The mixture was allowed to react for 1h at 90°C to obtain complete derivatisation. The reaction was monitored by TLC analysis eluting a silica plate with diethylether. Under these conditions only one spot (bis-(trimethylsilyl)-5-bromo-2′-deoxyuridine) could be demonstrated (R_f: 0.8) (Quilliam and Westmore, 1977). Solvents were removed by a stream of dry N_2 under mild heating (40–50°C) and the oily residue was redissolved in 400 μL dry THF. Mass spectromety was applied for final identification. One μL of the solution of the trimethylsilyl compound was brought on a tungsten wire by means of a micro syringe. After evaporation of the solvent the DCI probe was inserted into the ion source. The wire was heated at a rate of 9 mA/s until protonated molecular ions MH^+ were observed (BET $\pm$ 200 mA). DCI-mass spectra were recorded on Riber 10-10B-quadrupole mass spectrometer (Nermag S.A.) equipped with a Sidar Data System. Primary ionisation of the reagent gas (NH_3) was done by 70 eV electrons (I_s: 0.08 mA). The ion source pressure was 0.1 mmHg. Only the 3′-5′-*o*-bis-(trimethylsilyl)-5-bromo-2′-deoxyuridine (BSBDU) could be detected (Fig. 2).

Preparation of [^{11}C]thymidine (Fig. 3). The reaction flask A (see Fig. 4) containing the previously prepared BSBDU solution in THF was connected to the sodalime/P_2O_5 trap of the [^{11}C]methyl iodide production unit. This flask was cooled to -50°C under a flow of dry He. Two equivalents (32.6 μmol) n-butyl lithium were added to the solution and the mixture was stirred for 8 min. [^{11}C]Methyl iodide was then instilled and after 10 min of additional stirring the reaction was stopped and the protecting groups removed by addition of 400 μL 1M HCl, warmed up to room temperature, and finally the mixture was neutralised to pH 7 with 1M NaOH and pumped into flask B, filled with 500 μL chloroform. The aqueous phase was further purified by HPLC on a C_{18} HL 30 μ column (25 × 1 cm i.d.) (RSL, Eke, Belgium). The column was eluted with 3% (v/v) ethanol in an aqueous buffer of 0.05 M sodium phosphate and phosphoric acid (pH 4) at a flow rate of 4 mL/min (LKB 2150 pump, Bromma, Sweden) resulting in a back pressure of 20 bar. The effluent was monitored

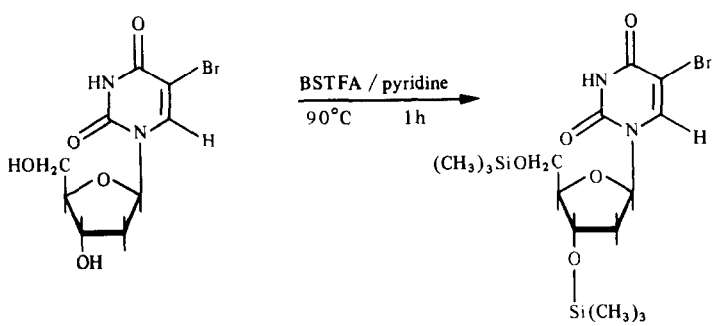

Fig. 1. Synthesis of 3′,5′-bis-(trimethylsilyl)-5-bromo-2′-deoxyuridine.

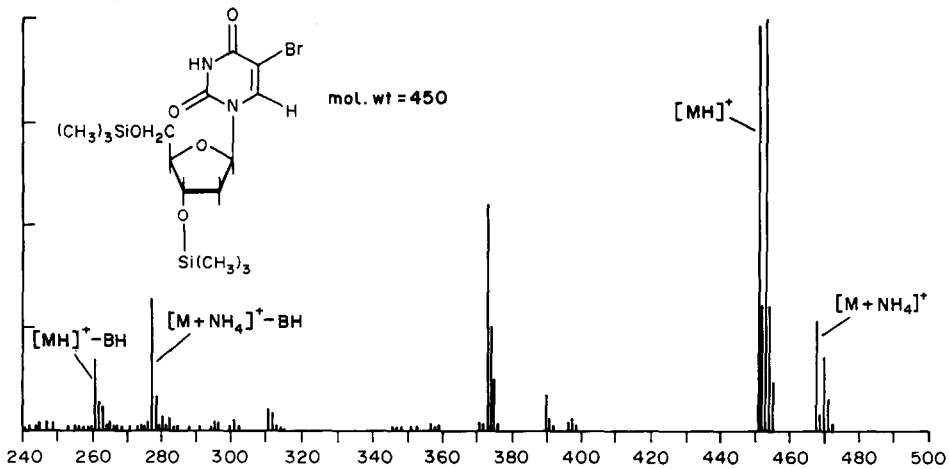

Fig. 2. Mass spectrum of 3′,5′-bis-(trimethylsilyl)-5-bromo-2′-deoxyuridine.

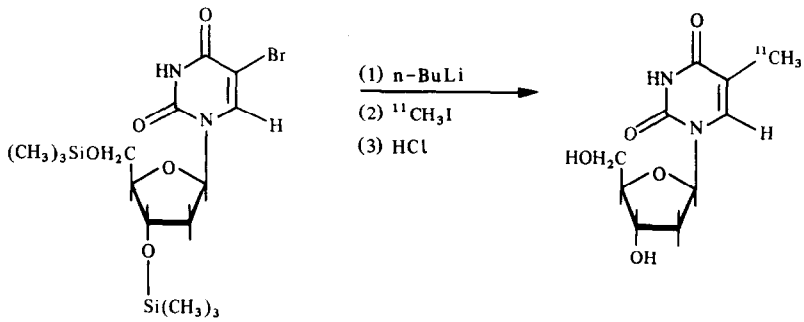

Fig. 3. Synthesis of [^{11}C]thymidine.

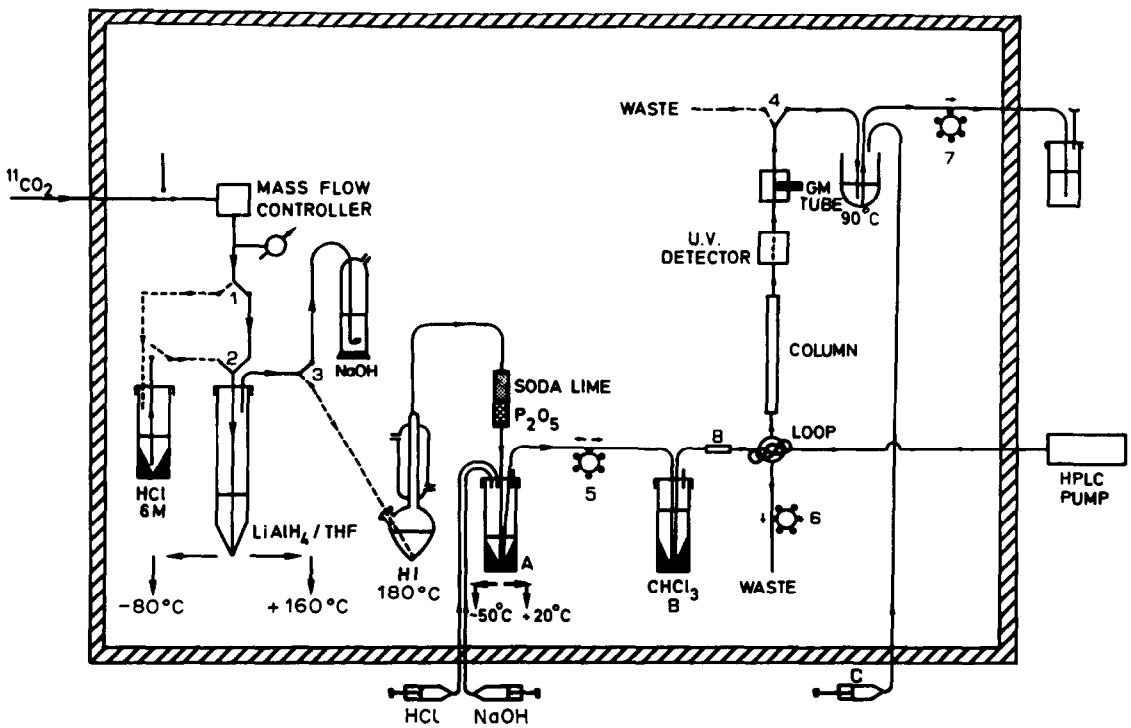

Fig. 4. Technical set-up for the production of [^{11}C]thymidine. (A) Reaction mixture for synthesis of [^{11}C]thymidine; (B) washing flask; (C) phosphate. 1–2–3–4, Electromagnetic 3-way valves; 5–6–7, peristaltic pumps; 8, filter.

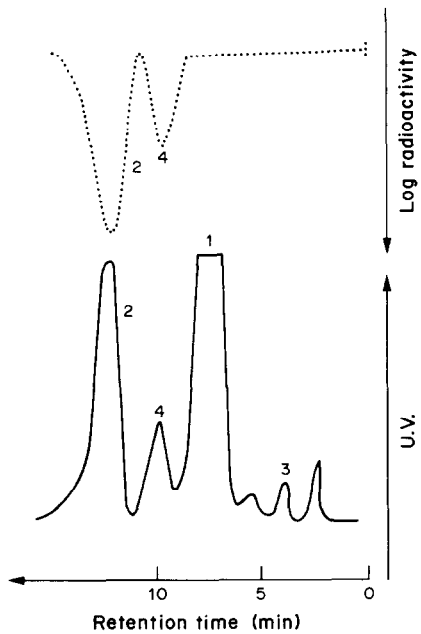

Fig. 5. Preparative radiochromatography. (1) 2′-deoxyuridine; (2) [^{11}C]thymidine; (3) Uracil; (4) unknown by product.

simultaneously at 254 nm (LC_3 u.v. detector, Pye Unicam, Cambridge, U.K.) and by a GM-tube. The radiochromatogram is shown in Fig. 5. By means of an electromagnetic valve (4) only the effluent fraction containing [^{11}C]thymidine was collected in the evaporation vessel.

Results and Discussion

Langström reported a procedure where a solution of the lithium salt of tris(trimethylsilyl)-5-bromo-2′-deoxyuridine in THF was treated with [^{11}C]methyl iodide in the presence of complexing diamines or HMPA (Langström, 1980). We synthesised the 3′,5′-bis-(trimethylsilyl)-5-bromo-2′-deoxyuridine using BSTFA, a more powerful silylating reagent (Gehrke and Patel, 1977) instead of the bis-(trimethylsilyl)-acetamide(BSA)-chlorotrimethylsilane (TMCS) mixture as proposed by Langström to produce tris-(trimethylsilyl)-5-bromo-2′-deoxyuridine. This needed a shorter reaction time and resulted in a purer reaction product since the by-products of BSTFA are more volatile than those of BSA and, therefore, more easily removed (Nicholson, 1978). Moreover, BSTFA is less toxic than BSA (Lenga, 1985). TMCS was not used since it is corrosive and carcinogenic (Lenga, 1985) and could, if not completely removed, compete with [^{11}C]methyl iodide during the alkylating reaction (Bergström, 1982) and disturb the final HPLC purification. The synthesis of a tris-(trimethylsilyl) derivative (Langström *et al.*, 1981) should be avoided since the presence of a trimethylsilyl group at O-4 could cause silicon shift from O-4 to C-5 preventing alkylation on C-5 (Bergström, 1982) and gives pronounced deterioration of the reactivity of the organolithium compound (Massé, 1970). The trimethylsilyl groups are sufficiently stable as hydroxyl protecting groups to resist strong bases such as n-butyl lithium and are much easier to remove than the tetrahydropyranyl groups used by Sundoro-Wu *et al.* (1984). Treatment of BSBDU with 2 equivalents of n-butyl lithium and [^{11}C]methyl iodide yielded [^{11}C]thymidine and 2′-deoxyuridine. Raising the temperature from −78°C (Langström, 1980; Sundoro-Wu *et al.*, 1984) to −50°C during lithiation and alkylation resulted in higher yields and shorter reaction times, without generating more by-products. Dry *N*′,*N*-dimethylpropylurea (DMPU) can be added to the reaction mixture as cosolvent to promote the reaction (Seebach *et al.*, 1982). It is however very difficult to obtain completely anhydrous DMPU due to its hygroscopic properties. Consequently addition of DMPU often resulted in a drastic decrease of the reaction yield. It is not necessary to add carrier methyl iodide as suggested by Sundoro-Wu *et al.* (1984). The presence of sparteine instead of HMPA in the reaction mixtures (Langström, 1980) did not give a significant increase of the reaction yield and interfered with the HPLC purification. The alkylation reaction was stopped and the protecting groups removed by raising the temperature and adding a dilute HCl solution. Water (Langström *et al.*, 1981) gave incomplete hydrolysis. After neutralising, the aqueous mixture was washed with chloroform to remove the excess reagents and almost all by-products. HPLC analysis of the aqueous phase gave a radioactive peak with the same retention as a commercial sample of thymidine (Fig. 5, peak 2). The identity of the labelled compound was confirmed with TLC (Silica, diethylether/methanol 90/10) (Sundoro-Wu *et al.*, 1984) and i.r. analysis. Peak 1 corresponds with a standard of 2′-deoxyuridine. The unknown radioactive peak is believed to be [^{11}C]isothymidine (Massé, 1970). Starting from [^{11}C]methyl iodide, 20–30 mCi of [^{11}C]thymidine were produced in 25 min with a radiochemical yield of 40–70% and high radiochemical purity. The specific activity was calculated to be 20–30 mCi/μmol at the end of the synthesis.

Acknowledgements—This work was supported through a Grant of the Belgian Government (Geconcerteerde Onderzoeksactie OOA 85/90). We wish to thank Dr E. Esmans for running the mass spectrum and for his helpful discussions, and E. Gillis for technical assistance.

References

Bergstrom D. E. (1982) Organometallic intermediates in the synthesis of nucleoside analogs. *Nucleos. Nucleot.* **1**, 1.

Crawford E. J., Christman D., Atkins H., Friedkin M. and Wolf A. P. (1978) Scintigraphy with positron-emitting compounds. I. Carbon-11 labeled thymidine and thymidylate. *Int. J. Nucl. Med. Biol.* **5**, 61.

Christman D., Crawford E. J., Friedkin M. and Wolf A. P. (1972) Detection of DNA synthesis in intact organism with positron emitting methyl-^{11}C thymidine. *Proc. Natl. Acad. Sci. U.S.A.* **69,** 988.

Denutte H. R., Vandewalle T., Cattoir H. J., Vandecasteele C., Jonckheere J. A., Slegers G., Gelijkens C. F. and De Leenheer A. P. (1982) The preparation of ^{11}C-labeled caffeine. *J. Labelled Compd. Radiopharm.* **19,** 735.

Gehrke C. and Patel A. (1977) Gas–liquid chromatography of nucleosides. Effect of silylating reagents and solvents. *J. Chromatogr.* **130,** 103.

Hampton A., Chawla R. and Kappler F. (1982) Species- or isoenzyme-specific enzyme inhibitors 5. Differential effects of thymidine substituents on affinity for rat thymidine kinase isozymes. *J. Med. Chem.* **25,** 644.

Langström (1980) Ph.D. Thesis. (University of Uppsala, Sweden).

Langström B., Sjoberg S., Bergson G., Lundqvist H., Malmborg P., Stalinacke C.-G. and Larsson B. (1981) ^{11}C methyl iodide in the synthesis of ^{11}C-compounds. *J. Labelled Compd. Radiopharm.* **18,** 17.

Lenga R. E. (1985) Safety. *The Sigma–Aldrich Library of Chemical Safety Data.*

Massé B. (1970) Ph.D. Thesis (Université de Lyon, France).

Nicholson J. D. (1978) Derivative formation in the quantitative gas-chromatographic analysis of pharmaceuticals. *Analyst* **103,** 193.

Plunkett W. and Cohen S. S. (1977) Penetration of mouse fibroblasts by 2′-deoxyadenosine 5′-phosphate and incorporation of the nucleotide into DNA. *J. Cell Physiol.* **91,** 261.

Pichat L., Massé B., Deschamps J. and Dufay P. (1971) Lithiation de la bromo-5-disoxyuridine trimethylsilylée. Méthode de préparation de thymidine (methyl ^{14}C). Obtention simultanée de méthyl-6-désoxyuridine. *Bull. Soc. Chim. Fr.* 2102.

Quilliam M. and Westmore J. B. (1977) Sterically crowded trialkylsilyl derivatives for chromatography and mass spectrometry of biologically important compounds. *Anal. Chem.* **50,** 59.

Seebach D., Henning R. and Mukhopadhyay T. (1982) Doubly deprotonated methyl 3-nitropropanoate an acrylic ester d^2-reagent. *Chem. Ber.* **115,** 1705.

Sundoro-Wu B., Schmall B., Conti P. S., Dahl R. J., Drumm P. and Jacobssen J. K. (1984) Selective alkylation of pyrimidyl dianions. Synthesis and purification of ^{11}C-labeled thymidine for tumor visualization using positron emission tomography. *Int. J. Appl. Radiat. Isot.* **35,** 705.