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III. 1 Automated Synthesis System for Production of [^{11}C] Fatty Acid with Computer Control

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Introduction

Fatty acids are the primary substrate for aerobic myocardial metabolism and labeled fatty acids have been used to study myocardial metabolism in vitro and in vivo in animals and man.¹⁾ [^{11}C]Palmitic acid have been used for the quantitative measurement of both regional myocardial perfusion and regional fatty acid metabolism with a PET imaging system.²⁻¹⁰⁾ 3-Methyl [^{11}C]heptadecanoic acid, a fatty acid analog designed to inhibit the β -oxidation process by preventing the formation of the corresponding β -ketoacyl SCoA, has also had the basic properties required for potential use in the assessment and quantitation of free fatty acid metabolism in the heart.¹¹⁾ In general, synthesis of short-lived radiopharmaceuticals needs a large quantity of starting radioactivity and therefore the radiation dose to the chemist is appreciable. [^{11}C]Fatty acid is easily synthesized by the reaction of $^{11}\text{CO}_2$ with the corresponding alkyl magnesium bromide, but the actual preparation for routine study entails several additional steps of isolation, binding of the labeled fatty acid to albumin and membrane filtration. To accommodate the increasing needs of these [^{11}C]labeled fatty acids in routine medical diagnosis study, remote-controlled synthesis system have been developed. Several remote-controlled synthetic methods have been reported.¹²⁻¹⁴⁾ We describe here the more advanced automated synthesis system with minimum radiation exposure to the chemist.

Materials and Methods

Chemicals: 1-Bromopentadecane (1a) and palmitic acid (2a) were purchased from Tokyo Kasei Kogyo Co., Ltd.

Chromatography: Thin-layer chromatography (TLC) was done on silica gel plates using the solvent indicated. High-performance liquid chromatography (HPLC) was carried out using a reversed-phase column (Fatty acid analysis (WATERS), 8-10 μm , 3.9 mm I.D. \times 30 cm long) with THF/MeCN/ H_2O (5/9/7) as solvent (flow rate: 1.0 ml/min).

(Synthesis of Starting Materials)

Synthesis of 1-bromo-2-methyl hexadecane (1b): 1b was synthesized according to the previous report.^{11,15} b.p. 130-135°C/0.6 mmHg (lit. 131°C/1.2 mmHg), MS (M^+-Br): 239, TLC Rf=0.92 (developing solvent: hexane/ether=4/1).

General preparation of Grignard reagents: The reaction vessel initially contained dry Mg (100 mg) and a small crystal of iodine. Air was purged from the apparatus by a stream of Ar. Under Ar atmosphere, a small portion of solution of alkyl bromide (1 g) dissolved in sodium-dried THF (10 ml) was run in and the vessel warmed gently until the color of iodine disappeared. After reaction has started, moderate reflux is maintained by addition of rest solution of alkyl bromide dissolved in sodium-dried THF. Reflux was continued for 90-180 min under an Ar atmosphere. The reaction mixture was cooled to room temperature and 5 ml of the solution was injected into the $^{11}CO_2$ trapping vessel.

(^{11}C -labeling)

Production of $^{11}CO_2$: The $^{11}CO_2$ was produced via the $^{14}N(p,\alpha)^{11}C$ reaction by proton bombardment (18 MeV, 10 μA) of a 24 kg/cm² N₂ gas target using the CGR-MeV model 680 Cyclotron of Tohoku Univ. A 60 min irradiation gave $^{11}CO_2$ in 640 mCi at the end of bombardment.

Emulsification of [^{11}C]fatty acid for injection: To the ethanol solution of [^{11}C]fatty acid supplied with automated synthesis system, 1 ml of ethanol containing 0.25 ml of Tween 20 was added and the solvent was evaporated in vacuo. To the residue, 1 ml of an aqueous solution of albumin (bovine) (2g/100 ml) was added with shaking at 60°C and the emulsion was filtered through a 0.22 μm pore size membrane filter for injection.

Purity determination: The radiochemical purity was determined by radio-HPLC. Retention time: C₁₅H₃₁¹¹COOH (8.6 min.), C₁₄H₂₉CH(CH₃)¹¹COOH (8.7 min.). [^{11}C]Palmitic acid was >99% pure, 3-methyl[^{11}C]heptadecanoic acid was >94% pure.

(Automated synthesis)

Synthetic method of [^{11}C]fatty acid: The ^{11}C -labeling scheme of fatty acid is shown in Fig. 1. The synthetic procedure used consists of the following steps:

- (1) Collecting of $^{11}CO_2$ in THF at room temperature with a flow rate of 200 ml/min.
- (2) Reaction of collected $^{11}CO_2$ with Grignard reagent by He bubbling (flow rate: 100 ml/min.)
- (3) Injection of 2N-HCl (2 ml) and hexane (4 ml) in reaction vessel.
- (4) Hydrolysis and extraction by He bubbling (flow rate: 100 ml/min.).
- (5) Transfer the extract to SEPALTOR.
- (6) Injection of fresh hexane (Ca. 10 ml) in reaction vessel.
- (7) Rinse of reaction vessel.

- (8) Transfer the washings to SEPALTOR.
- (9) Adsorption of produced [^{11}C]fatty acid on SEP-PAK (silica gel).
- (10) Elution of [^{11}C]fatty acid from SEP-PAK with ethanol (Ca. 10 ml).

Automated synthesis system: The system flow chart is shown in Fig. 2. The controller consists of a microcomputer (Hewlett Packard model 9826A, 320K bytes, BASIC), a graphic printer (Hewlett Packard model 2671 G), a color CRT, interfaces and a radioactivity monitor (Therados direct patient dose monitor model DPD-5). The current step procedure in the flow chart is displayed on the color CRT and the current step number, elapsed time and monitored radioactivity is displayed on the internal CRT of the computer. The system interface drives solenoid valves by a time-sequence program and received signal from optical liquid level sensors. Three optical liquid level sensors are applied for detecting an existence of liquid material in glassware from outside. The synthesis unit is supplied with a pressurized He gas for transferring liquid materials. The reaction or extraction is done with agitation by He bubbling. For extraction, SEPALTOR (Kokubo Seiki Co., Ltd.) is applied, which separation is accomplished by passing only the nonpolar solvent (hexane) from water-hexane mixture through a porous teflon membrane attached to the center of the device. A teflon tube (i.d. 1.0 mm) and teflon solenoid valves are used for controlling liquid material transfer in the synthesis unit. Each step can be manually paused during the automated synthesis. Individual solenoid valves can also be manually operated. Figure 3 shows photographic views of the main part of the system. The synthesis unit is housed within the shielded box neighboring the operation console.

Set-up procedure: The specially designed SEPALTOR and dehydration column packed with fresh anhydrous MgSO_4 are connected with the synthesis unit and SEP-PAC (silica gel) is then attached and 2N-HCl (2 ml)-hexane (4 ml), hexane (Ca. 10 ml) and ethanol (Ca. 10 ml) are charged in the corresponding reservoirs. Finally the dried reaction vessel is connected with the synthesis unit.

Automated synthesis of [^{11}C]fatty acid: After checking no pressure decrease in the synthetic unit and injection Grignard reagent by manual, the computer starts to control the synthetic procedure consisting of the 10 steps shown in Fig. 4. After the end of synthesis, it reports the results of the automated synthesis conditions.

Results and Discussion

As shown in Fig. 3, the synthetic unit is portable and can be easily and quickly removed from the shielded box.

Figure 4 shows the flow chart of synthetic procedure. Step 3, Step 6 and Step 10 are controlled by the optical liquid level sensors and other steps by a time-sequence.

Figure 5 shows typical computer displays of the radioactivity curves on

the reaction vessel. Part (A) displays the collecting ^{11}C radioactivity curve on the reaction vessel. Using this relative radioactivity value, an approximately yield of [^{11}C]fatty acid could be estimated. After hydrolysis (Step 4), a large activity loss (Part (B)) is due to releasing $^{11}\text{CO}_2$, which unreacted and only dissolved in THF. Part (C) displays the decreasing curve of radioactivity transferred from reaction vessel to SEPALTOR at Step 5.

SEPALTOR has been effective for the procedure of extraction, which is troublesome to automate. It has proved suitable for automatic synthesis.

At emulsification procedure, complete removal of ethanol is important to prevent the precipitating of denatured albumin. The time required for emulsification was 5-10 min.

As shown in Fig. 6, the synthesis of [^{11}C]fatty acid was thus completely automated. It was usually carried out within 30 min after the end of bombardment. This system has been used for more than 20 preparations of [^{11}C]palmitic acid and more than 20 preparations of 3-methyl [^{11}C]heptadecanoic acid. The experimental data of [^{11}C]fatty acids syntheses are summarized in Table 1. The supplied yield of [^{11}C]palmitic acid has been 20-30 mCi and that of 3-methyl [^{11}C]heptadecanoic acid has been 2-7 mCi. The lower radiochemical yield of 3-methyl [^{11}C]heptadecanoic acid may be due to steric hindrance caused by the methyl group at 3-position.

Use of the automated synthesis system described herein allows for the preparation of reasonable amounts of [^{11}C]fatty acid. This system has been confirmed to be suitable for routine production and the shielded automated system also accomplishes the most necessary function of reducing the radiation exposure to the operator.

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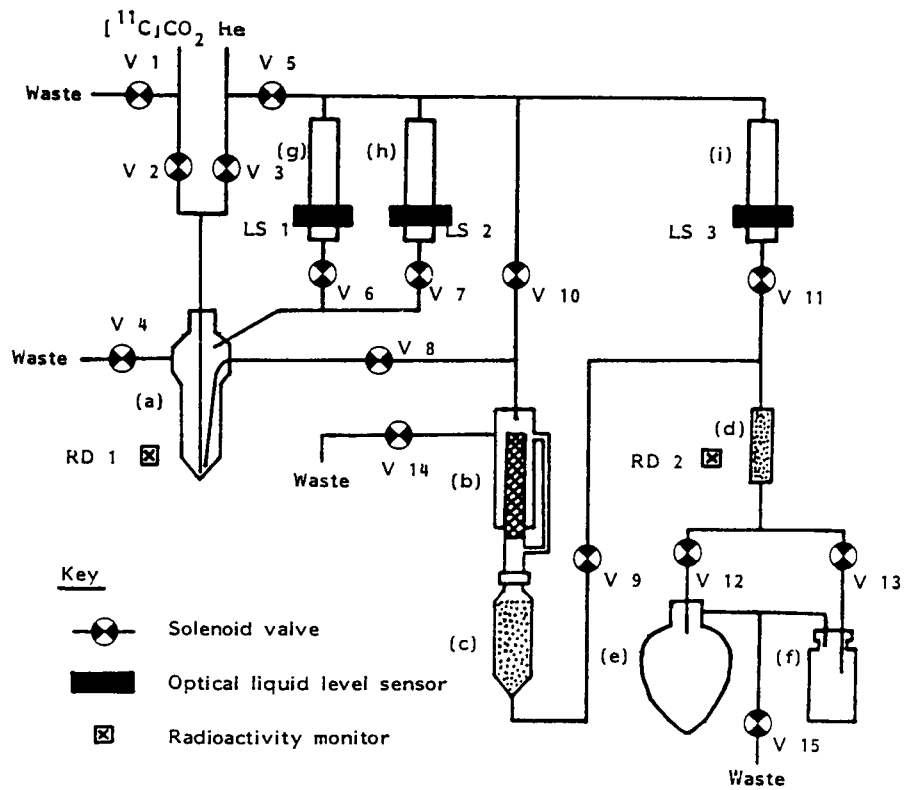


Fig. 2. System flow chart. (a) Reaction vessel, (b) SEPALTOR, (c) Anhydrous MgSO_4 , (d) SEP-PAK (silica gel), (e) Product reservoir, (f) Waste solvent reservoir, (g) 2N-HCl + hexane, (h) Hexane for rinsing, (i) Ethanol.

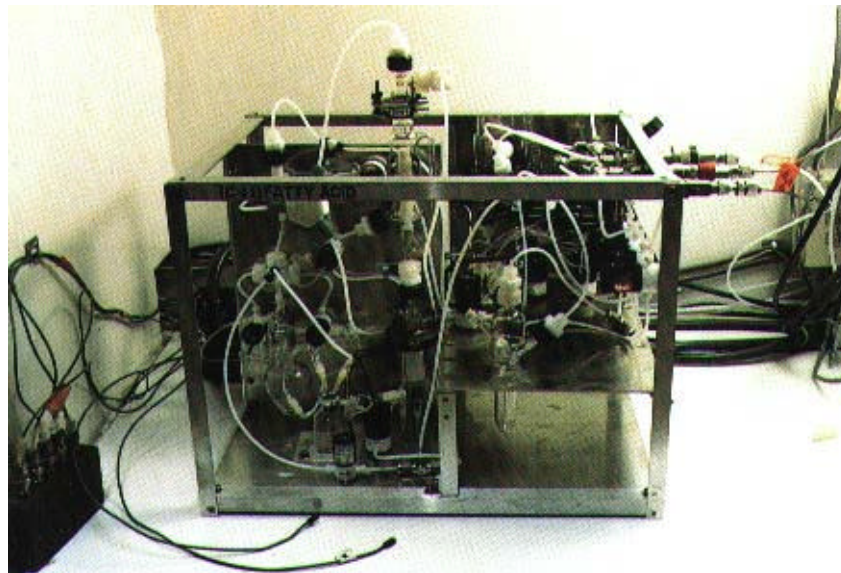


Fig. 3. Photographic views of the main part of the system. Synthesis unit in the shielded box.

Step procedure	Using valve No.	Using sensor No.	Stepping method
Leak test	2, 4	—	Manual
Injection of Grignard reagent	3, 4	—	Manual
1) Trapping of [^{11}C]CO ₂	2, 4	RD 1	Auto (15 min)
2) Reaction of [^{11}C]CO ₂ with Grignard reagent	1, 3, 4	RD 1	Auto (3 min)
3) Injection of 2N-HCl and hexane	4, 5, 6	RD 1, LS 1	Auto (LS 1)
4) Hydrolysis and extraction	3, 4	RD 1	Auto (2 min)
5) Transfer to SEPALTOR	3, 8, 14	RD 1	Auto (1 min)
6) Injection of fresh hexane	4, 5, 7	RD 1, LS 2	Auto (LS 2)
7) Rinse of reaction vessel	3, 4	RD 2	Auto (1 min)
8) Transfer to SEPALTOR	3, 8, 14	RD 2	Auto (1 min)
9) Adsorption on SEP-PAK (silica gel)	5, 9, 10, 13, 15	RD 2	Auto (3 min)
10) Elution of [^{11}C]fatty acid from SEP-PAK	5, 11, 12, 15	RD 2, LS 3	Auto (LS 3)

RD : Radioactivity detector , LS : Optical liquid level sensor

Fig. 4. Flow chart of synthetic procedure.

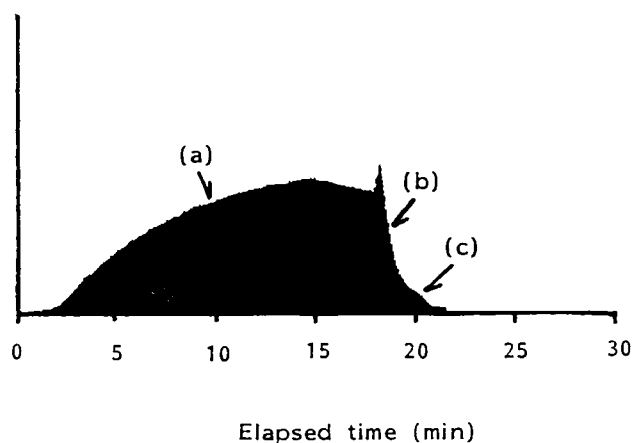


Fig. 5. Typical computer displays. Radioactivity on the reaction vessel.

SYNTHESIS

Total synthesis time : 10:47:56 - 11:14:55 (27.0 min)

Step procedure	Step elapsed time	Stepped by
1) Trapping of [¹¹ C]CO ₂	10:47:56 - 11:02:54 (15.0 min)	Auto
2) Reaction of [¹¹ C]CO ₂ with Grignard reagent	11:02:54 - 11:05:55 (3.0 min)	Auto
3) Injection of 2N-HCl and hexane	11:05:55 - 11:06:04 (.1 min)	Auto
4) Hydrolysis and extraction	11:06:04 - 11:08:00 (2.0 min)	Auto
5) Transfer to SEPALTOR	11:08:00 - 11:09:00 (1.0 min)	Auto
6) Injection of fresh hexane	11:09:00 - 11:09:21 (.3 min)	Auto
7) Rinse of reaction vessel	11:09:21 - 11:10:18 (1.0 min)	Auto
8) Transfer to SEPALTOR	11:10:18 - 11:11:18 (1.0 min)	Auto
9) Adsorption on SEP-PAK (silica gel)	11:11:18 - 11:14:18 (3.0 min)	Auto
10) Elution of [¹¹ C]fatty acid from SEP-PAK	11:14:18 - 11:14:55 (.6 min)	Auto

Fig. 6. Typical report of the automated synthesis.

Table 1. Experimental data of [¹¹C]fatty acid synthesis.

Compounds	Radiochemical yield ^{a)} (%)	Radiochemical purity (%)
C ₁₅ H ₃₁ - ¹¹ COOH	46 - 67	> 99
C ₁₄ H ₂₉ CH ₃ CHCH ₂ - ¹¹ COOH	10 - 25	> 96

a) % based on trapped [¹¹C]CO₂