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# Synthesis of [1-$^{11}$C]Octanoic Acid, [$^{11}$C]Raclopride and [$^{11}$C]Nicergoline with a General-purpose Automated Synthesis Apparatus of $^{11}$C-labeled Radiopharmaceuticals

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We have developed a general-purpose automated synthesis apparatus of $^{11}$C-labeled radiopharmaceuticals for PET, which can be adopted to both one-pot and two-or-more-pot reactions. The features of the apparatus were shown in the successful preparation of [1-$^{11}$C]octanoic acid in a one-pot reaction and [$^{11}$C]raclopride and [$^{11}$C]nicergoline in two-pot reactions, the latter being a novel radiopharmaceutical.


## Introduction

Positron emission tomography (PET) is a non-invasive quantitative imaging technique in which radioactive tracers labeled with positron emitters are used to measure *in vivo* the anatomical distribution of the radioactivity and rates of biochemical and physiological processes. A wide variety of positron-labeled compounds has been synthesized since PET proved to be an useful tool both in research and the clinical fields. Various types of synthesis apparatuses have also been developed for synthesizing positron-labeled compounds (Pike *et al.*, 1984; Hamacher *et al.*, 1990; Luthera *et al.*, 1994; Culbert *et al.*, 1995).

Considering the importance of carbon-11 labeling in PET, automated apparatuses have already been developed for the synthesis of [1-$^{11}$C]labeled aldoses (Nishimura *et al.*, 1994), [$^{11}$C]methyl iodide (Cork *et al.*, 1994) and [1-$^{11}$C]labeled carboxylic acids (Yajima *et al.*, 1995). As an extension of our study, we constructed this time a general-purpose automated synthesis apparatus, and tried by using it to prepare [1-$^{11}$C]octanoic acid, a potential radiotracer in PET-imaging of the brain functions (Kuge *et al.*, 1995), as an example of one-pot reaction and (S)-3,5-dichloro-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-[$^{11}$C]methoxysalicylamide ([$^{11}$C]raclopride), a labeled dopamine-$D_2$ ligand (Farde *et al.*, 1988) and (+)-[6-$^{11}$C]methyl-10-methoxy-1-methylergoline-8$\beta$-methanol 5-bromonicotinate ([$^{11}$C]nicergoline) as examples of the two-pot synthesis of C-11 labeled compounds. Nicergoline is well known as a drug which activates cerebral circulation (Moretti *et al.*, 1979) but has not been positron labeled yet.

## Materials and Methods

### *Reagents*

Commercially available reagents were purchased from Aldrich Chemical Co. Inc. (U.S.A.), Nacarai Tesque Inc., or Wako Pure Chemical Industries Ltd. THF was distilled over lithium aluminum hydride (LAH) in an argon atmosphere just before use. (-)-(S)-2-Aminomethyl-1-ethylpyrrolidine and desmethylnicergoline were purchased from Yoshitomi Pharmaceutical Industries Ltd, and Tanabe Seiyaku Co. Ltd, respectively. Authentic sample of raclopride was purchased from Funakoshi Co. Ltd and nicergoline was a gift from Tanabe Seiyaku Co. Ltd. A solution of heptyl bromide (14.33 g) in the dehydrated THF (100 mL) containing magnesium turnings (1.94 g) was heated under reflux in an argon atmosphere for 1 h to give a heptylmagnesium bromide solution in THF (*ca* 0.2 M), which was sealed in ampoules and kept at room temperature. Optically active desmetylraclopride was prepared

according to the method reported by Ehrin *et al.* (1987) with modification. Briefly, (-)-(S)-2-aminomethyl-1-ethylpyrrolidine was condensed with 3,5-dichloro-2,6-dimethoxybenzoic acid using N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride as condensing agent in the presence of N-hydroxybenzotriazole giving optically active 2-(3,5-dichloro-2,6-dimethoxybenzoylaminomethyl)-1-ethylpyrrolidine in 77% yield. Cleavage of the two methoxy groups of the latter compound by the two-step procedure of Ehrin *et al.* (firstly with $BBr_3$ and then with HBr/acetic acid), gave desmethylraclopride hydrobromide in 42% yield. No racemization was detected on a high-performance liquid chromatography (HPLC) analysis with an optically active column (Ultron ES-OVM, 4.6 × 150 mm).

*Automated synthesis apparatus*

Figure 1 shows the schematic diagram of the apparatus used for the one-pot reactions and Fig. 2 the one used for the two-pot reactions. The essential design concept is the same as that adopted in the construction of the previous apparatuses (Nishimura, Cork and Yajima, *loc. cit.*) with modifications made based on our own experience. Modifications adopted are as follows: (1) for easy assembly, (a) each reaction flask and the degas flask have one or two jackets and all heating/cooling operations are performed by circulating themostated (LAUDA) heating/cooling fluid through them. (b) The top of those flasks is sealed by a butyl-rubber septum and an aluminum cap, gases and liquids being brought in and out of the flasks through needles stung through the rubber septum. The needles are connected to Teflon-tubings each with a lure-locked connector. (2) For easy maintenance: the reaction unit (from Reg. 1 to V11) and the purification/formulation unit (the remaining parts) are mounted separately on two racks of the size of $^{W}300 \times {}^{D}300 \times {}^{H}600$ mm. Each rack has casters and can be turned freely even in a narrow place in a Hot Cell, when the cables joining the apparatus to the I/0 boards and the tubings between the two racks are disconnected. For evaporation a commercial micro-evaporator (Mitamura Riken Kogyo Inc.) is modified and used.

The operation of the apparatus is controlled with a personal computer (486DX2, NEC); an adapter (RS422) in the computer controlling I/0 boards, PAMUX (Opto22, U.S.A.). Six digital and two analogue brain boards, each with 16 I/O channels, are used. The digital out channels are for operating the solenoid valves (Takasago Electric Inc.), a $CO_2$ trap equipment (Sumitomo Heavy Industries Co. Ltd), the micro-evaporator, a HPLC pump (GULLIVER PU-987, Japan Spectroscopic Co. Ltd), magnetic stirrers, a 6-way valve, and so on. The digital input channels read the status of 16 indicator lamps that are used for monitoring photosensors and the position of the 6-way valve. The analog channels are for reading status information from the apparatus, including radioactivity of CsI scintillators, u.v. absorbance, pressure and gas flow-rates, and for writing information to set flow-rates of mass flow controllers (MARK III, Estec Inc.), and so on. The keyboard type auto/manual switch box which contains 80 indicator lamps/switches and 16 indicator lamps for digital boards is set between I/O boards and the apparatus.

[$^{11}$C]Carbon dioxide was produced via $^{14}N(p, \alpha)\ ^{11}C$ reaction using a cyclotron-target system (CYPRIS HM-18, Sumitomo Heavy Industries Co. Ltd). Radioactive impurities ($^{11}CO$, etc.) were removed by trapping [$^{11}$C]$CO_2$ under cooling with liquid argon. Transport of the reagents, solvents and reaction mixture was performed by argon gas pressure, except for the transfer of the [$^{11}$C] $CO_2$ which was performed by nitrogen gas pressure. After labeling reaction, the reaction mixture was transferred to a degas flask, and then, injected into the sample loop of the HPLC apparatus via the 6-way valve using argon gas pressure.

The HPLC columns used were: system 1: YMC-Pack ODS-AQ 250 × 20 mm I.D. and YMC-Guard Pack ODS-AQ 10 × 20 mm I.D.; system 2: YMC-Pack ODS-AQ 250 × 4.6 mm I.D. and YMC-Guard Pack ODS-AQ 10 × 4.0 mm I.D.; system 3: Capcell pak C-18, SG 120A 150 × 15 mm I.D. and Capcell pak C-18, 30 × 15 mm I.D.; system 4: Capcell pak C-18, SG 120A 250 × 4.6 mm I.D.; system 5: Ultron N C-18 150 × 4.6 mm I.D. For monitoring were used a u.v. detector, GULLIVER u.v.-970 (Japan Spectroscopic Co. Ltd) and radiodetectors, Positron Monitor TCS-R81-3454 (Aloka) and Gamma Detector FGD-102 (Aloka) together with Radio Analyser RLC-700 (Aloka).

*Preparation of* [1-$^{11}$*C*]*octanoic acid*

The reservoirs of the apparatus were filled as follows: reservoir 1 (RS1) (0.2 M $C_7H_{15}MgBr$/THF, 0.5 mL), RS 2 (2 N HCl, 0.5 mL), RS 3 (7% $NaHCO_3$ aq., 2.0 mL) and RS 4 ($CH_3CN$, 0.5 mL). After leak-check, the reaction flask and tubings were purged with argon gas (flow rate 30 mL/min). The position of the 6-way valve (6WV) was set in position 2. The Grignard solution was injected into the reaction flask (RF) from RS1 just before the start of the recovery of [$^{11}$C]$CO_2$. The [$^{11}$C]$CO_2$ was bubbled through the Grignard solution with $N_2$ gas (flow rate 16 mL/min) for 4 min. The hydrochloric acid in RS2 was injected into the reaction mixture to quench the reaction. Then, the reaction mixture was transferred to the degas flask and the reaction flask was rinsed with the $CH_3CN$. The reaction mixture and the washings were combined, mounted on the HPLC column. For the HPLC purification, HPLC system 1 was used with $CH_3CN$:$H_2O$:6N HCl = 550:500:1 (v/v) as eluent, flow rate: 20 mL/min; temperature: room temperature; retention time: *ca* 8 min and detection: u.v. ($\lambda$ 214 nm) and radioactivity. For quality control of the [1-$^{11}$C]octanoic acid was used

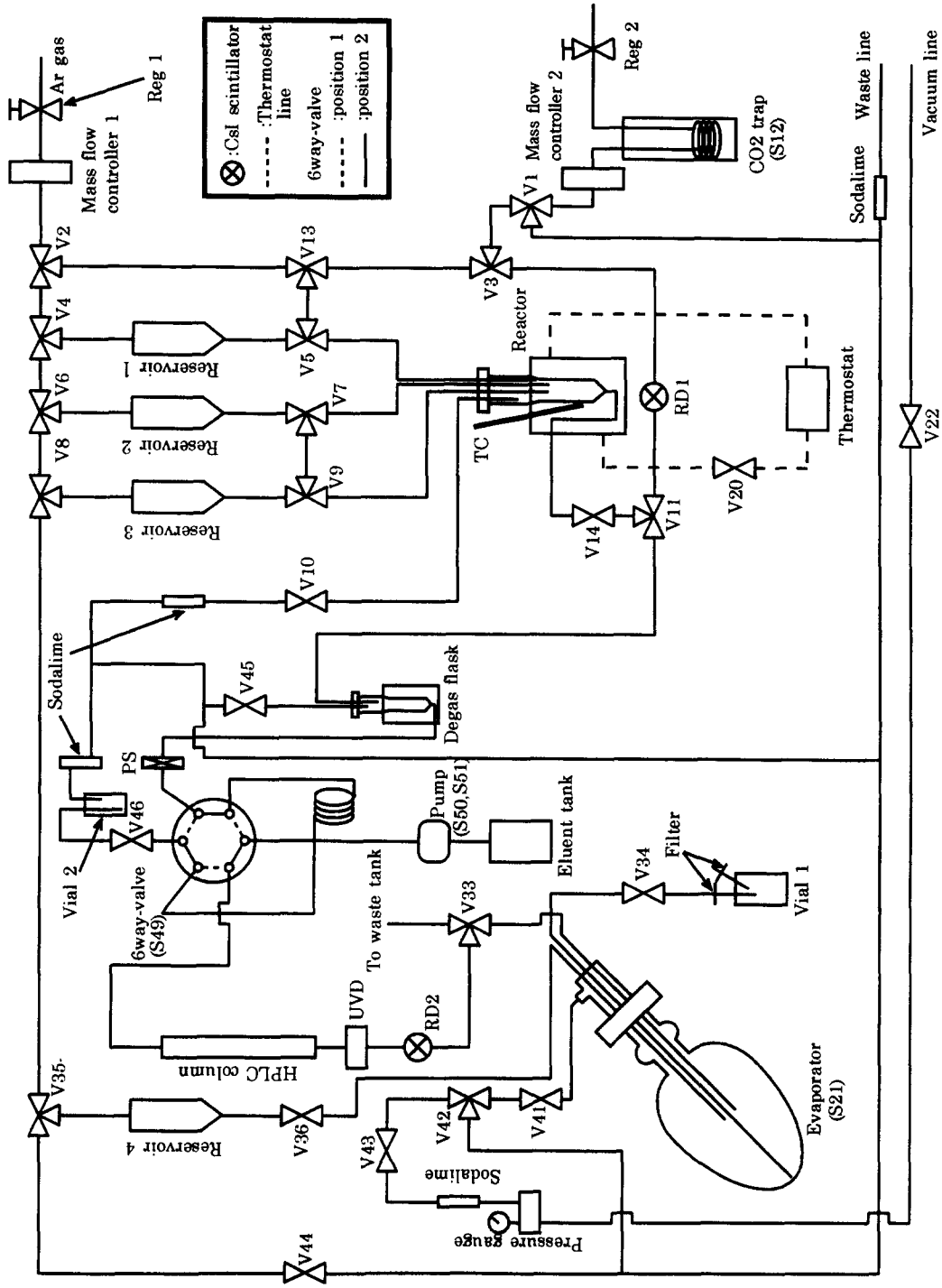



Fig. 1. Schematic diagram of the experimental apparatus for one-pot reaction. PS: photosensor; RD: radiodetector; Reg: regulator; TC: thermocouple; UVD: u.v. detector.

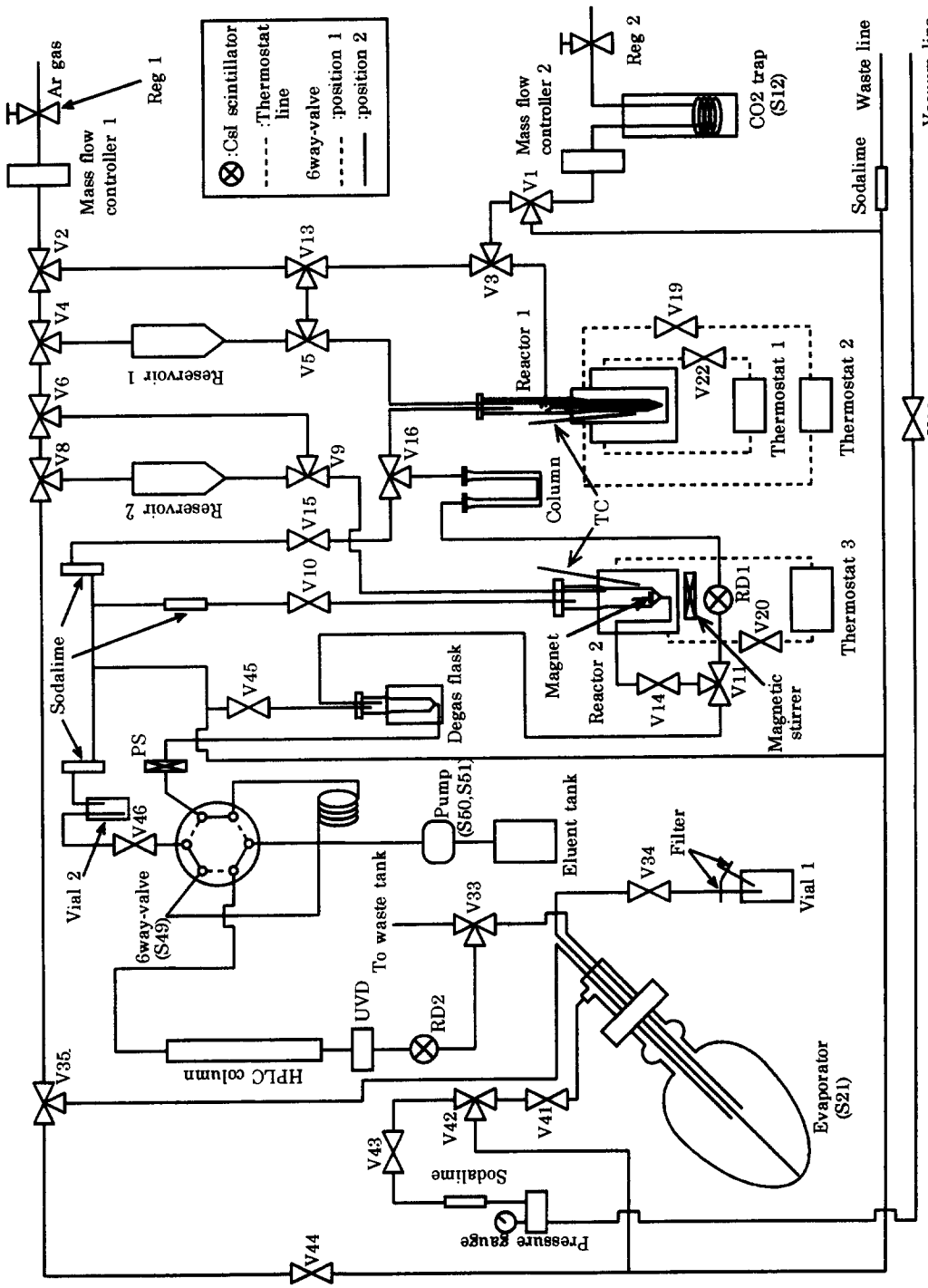
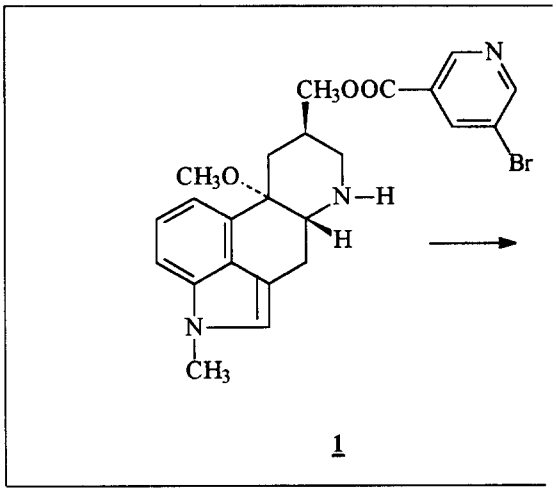



Fig. 2. Schematic diagram of the experimental apparatus for two-pot reaction. PS: photosensor; RD: radiodetector; Reg: regulator; TC: thermocouple; UVD: u.v. detector.

HPLC system 2 with $CH_3CN$:$H_2O$:6N HCl = 600:400:1 (v/v) as eluent, flow rate: 1.0 mL/min; temperature: room temperature; retention time: *ca* 12 min and detection: as above. The fraction containing [1-$^{11}$C]octanoic acid was collected in the evaporation flask by switching on a valve (V33). The solution collected was then neutralized with the 7% $NaHCO_3$ from RS4 and concentrated under reduced pressure. The residue was filtered through a millipore filter giving a ready-to-inject solution of the titled compound.

*Preparation of* [$^{11}$*C*]*raclopride*

The RSs of the apparatus in Fig. 2 were filled as follows: RS1 (47% HI, 0.4 mL) and RS2 ($CH_3CN$, 0.5 mL). A solution of LAH in THF (0.1 M, 0.5 mL) was injected into the reaction flask 1 and desmethylraclopride/DMSO (*ca* 4.5 μmol, 0.5 mL) and 5 M NaOHaq (10 μL) were injected into the RF2, respectively, using gas-tight syringes just before the start of the synthesis. Then, the $^{11}CO_2$ was bubbled through the LAH/THF solution with $N_2$ gas (flow rate 100 mL/min) from the cyclotron-target system for 1 min at − 20°C. Then, the HI was injected into the reaction mixture from RS1 and the mixture was heated up to 0°C within 1 min by stopping the cooling, and then by circulating through the jacket silicone oil heated at 130°C. [$^{11}$C]Methyl iodide formed was transferred to RF2 on a stream of argon gas at a flow rate of 100 mL/min through drying tubes until the radioactivity in RF2 reached a maximum. At the end of trapping of the [$^{11}$C]methyl iodide, RF2 was sealed by switching off valves V1, V10, V11, V13 and V14, the magnetic stirrer under the RF2 started and the flask heated at 85°C by switching on V20. After 5 min heating, the reaction mixture was transferred to HPLC column through the degas flask and the labeled objective product was separated and processed as before, giving a ready-to-inject solution of [$^{11}$C]raclopride.

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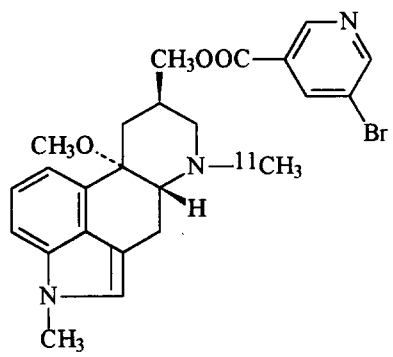
The HPLC conditions used for preparation were as follows: column: HPLC system 3 with MeCN:1.01 M $H_3PO_4$ = 25:75 (v/v) as eluent; flow rate: 10 mL/min; temperature: room temperature; retention time: *ca* 13 min and detection: u.v. (λ 214 nm) and radioactivity. For quality control of the [$^{11}$C]raclopride was used HPLC system 4 with MECN:0.01 M $H_3PO_4$ = 30:70 (v/v) as eluent; flow rate: 1.0 mL/min; temperature: room temperature; retention time: *ca* 10 min and detection: as above.

*Preparation of* [$^{11}$*C*]*nicergoline* (2)

The experimental procedure was similar to that for the preparation of [$^{11}$C]raclopride, except for that a solution of desmethylnicergoline (1)/DMF (*ca* 4.5 μmol/0.5 mL) and the N,N-diisopropylethylamine (10 μL) were placed in the RF2 instead of a mixture of desmethylraclopride/DMSO and NaOH aq.

The HPLC conditions used for preparation were as follows: columns: HPLC system 1 with 0.2% $NH_4H_2PO_4$:$CH_3CN$:EtOH = 51:30:19 (v/v) as eluent; flow rate: 20 mL/min; temperature: room temperature; retention time: *ca* 17 min and detection: u.v. (λ 280 nm) and radioactivity. For quality control of the [$^{11}$C]nicergoline was used HPLC system 5 with 0.2% $NH_4H_2PO_4$:$CH_3CN$:EtOH = 51:30:19 (v/v) as eluent; flow rate: 1.0 mL/min; temperature: room temperature; retention time: *ca* 20 min and detection: as before.

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## Results and Discussion

The apparatus was constructed as a general-purpose one not limited in its scope of applicability. It was composed of units for every basic operation, i.e. performing reaction, injection of HPLC column, collection of fractions, evaporation, and formulation, etc. In particular, we constructed the reaction, separation and purification units carefully based on our own experience. We adopted a batch or semi-batch type reaction system because of its reliability and generality. For heating/cooling of the reaction mixture, we adopted the method of circulating heating/cooling fluids through jackets attached to the reaction flasks. For the same purpose, blowing hot or cold gas is often used, because it is

Table 1. Production of the [$^{11}$C]labeled compounds

| Labeled compounds | Radioactivity[1] | Radiochemical purity | Specific activity |
|---|---|---|---|
| [1-$^{11}$C]octanoic acid[2] | 4.1–4.6 GBq | > 99% | — |
| [$^{11}$C]raclopride[3] | 10.4–17.4 MBq | > 99% | *ca* 18.5 GBq/μmol |
| [$^{11}$C]nicergoline[4] | 0.40–0.54 GBq | > 99% | *ca* 26.3 GBq/μmol |

[1]At the end of formulation.
[2]Irradiation: 15 μA × 40 min.
[3]Irradiation: 20 μA × 20 min.
[4]Irradiation: 20 μA × 40 min.

easy to switch heating to cooling and vice versa. It is rather difficult, however, to control the temperature precisely with the method. We used one or double jacketed Pyrex reaction flasks. It was possible for us to control the temperature of the reaction mixture at desired temperature of −30 to 180°C without significant delay by using the outer jacket for the initial setting of temperature and the inner jacket for the latter one.

We adopted HPLC method for purification, because it is most reliable and most universally applicable. The injection system adopted was the same as the one used in our previous work. It is composite of a degas flask, a photosensor and a 6WV and it worked without failure in more than hundred experiments using the system. The injection loss was less than 4%. The algorithm of automated fraction collection by monitoring the radiodetector was the same as the one described previously (Nishimura *et al.*, *loc. cit.*).

Another reservoir in use in the washing process of the apparatus is not shown in Fig. 1. The reservoir was added when repeated synthesis of a labeled compound (e.g. in case of [1-$^{11}$C]octanoic acid synthesis) became necessary, in order to minimize the operator's exposure to radiation. Some programs for the washing process are prepared. We can deal with alteration of the software and hardware in several minutes.

The present apparatus is of quite general constitution and useful for the synthesis of various $^{11}$C-labeled radiopharmceuticals, which we confirmed in the synthesis of the three labeled compounds described above by now. Table 1 shows the ranges of the amount of radioactivity, radiochemical purity and specific activity of those labeled products obtained. The yield of the radioactivity of [1-$^{11}$C]octanoic acid was four times as large as that in the previous study (Yajima *et al.*, *loc. cit.*). This may be attributed to the prolonged reaction time (from 2 min 30 s in the previous work to 4 min in the present one) based on the result of a kinetics study on the reaction of heptylmagnesium bromide with $CO_2$ (Yajima *et al.*, 1996) and reduced adhesion loss due to the simplified construction of the apparatus. We have not applied the present apparatus to the preparation of other [1-$^{11}$C]carboxylic acids than [1-$^{11}$C]octanoic acid yet. However, we are convinced that it will readily be used in preparation of different [1-$^{11}$C]labeled carboxylic acids.

The amount of the radioactivity of [$^{11}$C]raclopride obtained was small, even if the irradiation time was rather short. Many by-products were found in the reaction mixture. It was not possible to cool the reaction mixture so as to improve the trapping efficacy of [$^{11}$C]$CH_3I$ because of the high melting point of the solvent, DMSO (18.5°C). We tried to use solvents or mixture of solvents of a lower melting point without succeeding in finding any other solvent more suitable to the reaction than DMSO. On the other hand, in the case of [$^{11}$C]nicergoline synthesis, it was possible to cool the reaction mixture to the temperature as low as 5°C, which raised the trapping efficacy of [$^{11}$C]$CH_3I$ in the reaction mixture considerably. [$^{11}$C]Nicergoline was obtained in fair radiochemical yields.

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