

## High reactivity of [ $^{11}\text{C}$ ]CH<sub>3</sub>I with thiol group in the synthesis of C-11 labeled radiopharmaceuticals

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High reactivity of [ $^{11}\text{C}$ ]-methyl iodide ([ $^{11}\text{C}$ ]CH<sub>3</sub>I) with the thiol group was demonstrated with cysteamine and other compounds containing a thiol and another functional groups in each structure. The methylation of the thiol group in cysteamine with [ $^{11}\text{C}$ ]CH<sub>3</sub>I was very rapid at 0°C with no catalyst, and gave a high radiochemical yield and purity without any detectable by-product. Moreover, this reaction was not disturbed by the other functional groups, such as -NH<sub>2</sub>, -OH and -COOH in the same structure. This S-methylation reaction is very useful for producing a new radiopharmaceutical labeled with the short lived positron emitting nuclide C-11.

**Key words:** radiopharmaceutical, positron emitting nuclide,  $^{11}\text{C}$ , thiol group, labeling reaction

### INTRODUCTION

THE POSITRON EMITTING NUCLIDE is very useful in clinical nuclear medicine because of its high resolution in Positron Emission Computed Tomography (PET). On this basis, many radiopharmaceuticals labeled with positron emitting nuclide C-11 have been developed. Numerous C-11 labeled compounds have been synthesized by the methylation reaction with [ $^{11}\text{C}$ ]-methyl iodide ([ $^{11}\text{C}$ ]CH<sub>3</sub>I) as a precursor. Examples are receptor binding ligands,<sup>1-3</sup> 3-O-[ $^{11}\text{C}$ ]-methyl-glucose,<sup>4</sup> amino acids,<sup>5-8</sup> and amine analogs.<sup>9,10</sup> Each methylation reaction needs optimum labeling conditions such as labeling time, temperature, solvent and an appropriate catalyst for a good yield. These radiopharmaceuticals have been synthesized by N-methylation with [ $^{11}\text{C}$ ]CH<sub>3</sub>I, and thus the reaction conditions have since been studied systematically.<sup>11,12</sup>

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Thiol groups display high reactivity with alkyl halides.<sup>13</sup> As a radiolabeled alkyl halide for N-methylation, [ $^{11}\text{C}$ ]CH<sub>3</sub>I is readily available via an automated synthetic system. For a precursor containing a thiol group in its structure, the labeling reaction with [ $^{11}\text{C}$ ]CH<sub>3</sub>I requires only a one-step reaction. Except for the synthesis of S-[ $^{11}\text{C}$ ]-methionine, evaluating labeling conditions of the S-methylation reaction has not been hitherto documented. In addition, the effects of other functional groups in the same structure on a reaction between the thiol group and alkyl halide has not been discussed, although biogenic compounds have many functional groups, such as amino, hydroxy and carboxylic groups.

Our present study attempted to develop new C-11 labeled radiopharmaceuticals. Five compounds, containing a thiol and another functional group in each structure, were selected as model compounds for the [ $^{11}\text{C}$ ]-S-methylation reaction.

### MATERIALS AND METHODS

All the reagents were of guaranteed grade and used without recrystallization. Thin layer chromato-

graphy (TLC) was carried out with silicagel plate Kieselgel 60 (Art 5553, Merck Co. Ltd., Tokyo). High Performance Liquid Chromatography (HPLC; LC-4A, Shimadzu Co. Ltd., Tokyo) was performed with a refractive index detector and radioactivity detector. NMR spectra were taken by means of a JOEL PMX 60 (Nihon Bunko Co. Ltd., Tokyo) with tetramethylsilane as the internal standard.

#### *Production of [ $^{11}\text{C}$ ]CH $_3\text{I}$*

C-11 was produced via the  $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$  reaction with 11.3 MeV protons on a nitrogen gas target in an ultra compact cyclotron (CYPRIS Model 325, Sumitomo Heavy Industry Ltd., Japan). The target batch nitrogen gas was bombarded and the [ $^{11}\text{C}$ ] CO $_2$  produced was transported into an automated [ $^{11}\text{C}$ ]CH $_3\text{I}$  synthesis system (CUPID, Sumitomo Heavy Industry Ltd.). The radioligand, [ $^{11}\text{C}$ ]CH $_3\text{I}$ , was synthesized according to the method of Comar et al.<sup>14</sup> Briefly, [ $^{11}\text{C}$ ]CO $_2$  was first reacted with LiAlH $_4$  in a tetrahydrofuran solution in the reaction vessel. After evaporating the solvent, HI solution was added to the residue. The resulting [ $^{11}\text{C}$ ]CH $_3\text{I}$  was distilled at 80°C and subsequently trapped in acetone cooled by liquid CO $_2$  gas.

#### *Synthesis of authentic S-methylated compounds*

Five mercapto compounds (Table 3) were used in this study. The authentic compounds of S-methyl-mercaptopropionic acid and S-methyl-mercaptoethanol were commercially available. S-methyl-cysteamine, S-methyl-thioglycolic acid and S-methyl-thioglycerol were synthesized according to the following general procedures. Briefly, each thiol compound (0.1 mole) was dissolved in a mixture of 100 ml of methanol and 100 mL of 1 N NaOH. After cooling, 0.11 mole of CH $_3\text{I}$  was added to the mixture and stirred at 0°C for 10 min prior to adjusting to an optimum pH value. The solution was extracted with chloroform, dried, evaporated to dryness, and then the products were obtained by distillation in vacuo.

#### *S-methyl-2-mercapto-acetic acid*

bp. 81.0–83.5°C (2.5 mmHg). Yield 61.5%. Elemental analysis agreed with the calculated value for C $_3\text{H}_6\text{O}_2\text{S}$ : C, 33.97%; H, 5.70%. Found C, 34.05%; H, 5.78%. NMR (CDCl $_3$ ): (ppm) 2.15 (s, 3H), 3.08 (s, 2H), 7.93 (s, 1H).

#### *S-methyl-3-mercapto-3-deoxy-glycerol*

bp. 100.5–101.0°C (2.0 mmHg). Yield 51.0%. Elemental analysis agreed with the calculated value for C $_4\text{H}_{10}\text{O}_2\text{S}$ : C, 39.34%; H, 8.25%; S, 26.21%. Found C, 39.09%; H, 8.49%; S, 26.14%. NMR (CDCl $_3$ ): (ppm) 3.52 (m, 5H), 2.52 (d, 2H), 2.07 (s, 3H).

#### *S-methyl-cysteamine*

bp. 48.0–48.5°C (14.5 mmHg). Yield 47.3%. Elemental analysis agreed with the calculated value for C $_{10}\text{H}_{17}\text{NO}_3\text{S}_2$  (as tosylate (mp. 91.0–94.0°C)): C, 45.62%; H, 6.51%; N, 5.32%. Found C, 45.32%; H, 6.56%; N, 5.22%. NMR (CDCl $_3$ ): (ppm) 1.68 (s, 2H), 2.03 (s, 3H), 2.48–2.78 (m, 4H).

In addition, S,N,N-tri-methyl- and S,N,N,N-tetramethyl-cysteamine were synthesized<sup>15</sup> as authentic compounds of methylated cysteamine analogs, respectively.

#### *S,N,N-trimethyl-cysteamine*

bp. 51.0–51.5°C (18.0 mmHg). Yield 295 mg (11.4%). Elemental analysis was agreed with the calculated value for C $_5\text{H}_{14}\text{NSCl}$  (as hydrochloride; mp. 156.0–158.0°C): C, 38.59%; H, 9.07%; N, 9.00%. Found C, 38.41%; H, 9.27%; N, 9.01%. NMR (CDCl $_3$ ): (ppm) 2.03 (s, 3H), 2.17 (s, 6H), 2.46 (s, 4H).

#### *S,N,N,N-tetramethyl-cysteamine hydroiodide*

mp. 229.0–231.0°C. Yield 750 mg (58.0%). Elemental analysis agreed with the calculated value for C $_6\text{H}_{16}\text{NSI}$ : C, 27.60%; H, 6.18%; N, 5.36%. Found C, 27.79%; H, 6.33%; N, 5.34%. NMR (CDCl $_3$ ): (ppm) 2.06 (s, 3H), 2.37–2.43 (dd, 4H), 3.09 (s, 9H).

#### *Methylation reaction by [ $^{11}\text{C}$ ]CH $_3\text{I}$*

Labeling conditions were determined with cysteamine as a model compound. Labeling parameters were tested by varying the reaction time (30 sec–10 min) and labeling temperature (0–50°C) with  $1 \times 10^{-5}$  mole cysteamine in 0.25 mL of 1 N NaOH and 0.1 mL of acetone containing [ $^{11}\text{C}$ ]CH $_3\text{I}$ . After the removal of non-reacted [ $^{11}\text{C}$ ]CH $_3\text{I}$ , the mixture was analyzed by TLC with silicagel plates and HPLC with a reverse-phase column C-18 at a column temperature of 40°C. The developing solvent for TLC was chloroform/methanol/ammonia water=8/2/1. The eluting HPLC solvent was acetonitrile/water/methylamine=49/50/1 and the flow rate was 0.5 mL/min. The proportion of the product was calculated from the area of the radioactive peak on the TLC or HPLC chart. Data are presented as the mean for every 3 trials. Using the cold synthesized compounds, the developed TLC showed R $_f$  values of 0.17–0.43, 0.50–0.71 and 0.00–0.10 for S-methyl-, S,N,N-tri-methyl- and S,N,N,N-tetramethyl-cysteamine, respectively. Retention time (R $_t$ ) HPLC intervals for S-methyl-, S,N,N-tri-methyl-, S,N,N,N-tetra-methyl-cysteamine and CH $_3\text{I}$  were 5.6, 7.7, 4.2 and 8.4 min, respectively.

The carrier effect of CH $_3\text{I}$  ( $3 \times 10^{-5}$  and  $1 \times 10^{-4}$  mole) was also tested in the manner described above. Non-radioactive CH $_3\text{I}$  was added in the acetone solu-

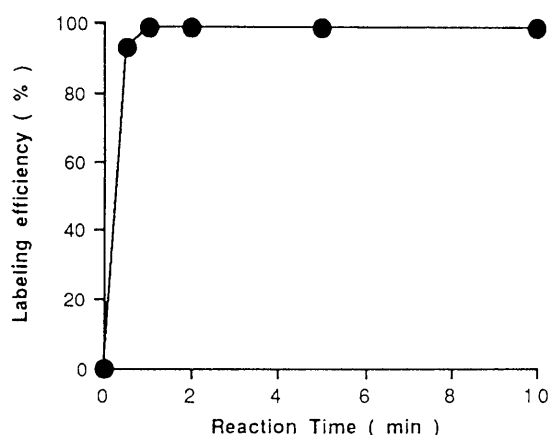
tion containing  $[^{11}\text{C}]\text{CH}_3\text{I}$ .

The methylation of the other four compounds by  $[^{11}\text{C}]\text{CH}_3\text{I}$  was carried out under the optimal conditions determined previously with cysteamine. Structures of the labeled compounds were confirmed by the respective  $R_f$  and  $R_t$  values for TLC and HPLC.

## RESULTS

### Methylation of cysteamine by $[^{11}\text{C}]\text{CH}_3\text{I}$

Various labeling parameters were first surveyed. As no differences were observed between 0 and 50°C, the labeling temperature of 0°C was selected to prevent the vaporization of  $[^{11}\text{C}]\text{CH}_3\text{I}$  in finding the optimum reaction time. At this temperature, a 93% yield was obtained after only 30 sec mixing, and plateaued after a 1-min reaction (Fig. 1). The following optimal conditions were selected:  $1 \times 10^{-5}$



**Fig. 1** Effect of reaction time on the S-methylation efficiency of cysteamine by  $[^{11}\text{C}]\text{CH}_3\text{I}$ . Labeling condition: cysteamine 1.14 mg ( $1 \times 10^{-5}$  mole) in 0.25 mL of 1 N NaOH and 0.1 mL of acetone (including  $[^{11}\text{C}]\text{CH}_3\text{I}$ ) at 0°C.

mole cysteamine was dissolved in 0.25 mL of 1 N NaOH at 0°C before adding 0.1 mL of acetone containing  $[^{11}\text{C}]\text{CH}_3\text{I}$ , and the mixture was stirred at 0°C for 1 min. The proportion of the desired compound was over 98% assayed by TLC and HPLC. The radioactive peak detected with TLC was superimposed over the stained spot of the authentic compound by iodide. The radioactive peak detected with HPLC indicated an  $R_t$  similar to that of the authentic compound.

Ethanolamine was  $[^{11}\text{C}]$ -methylated under the same labeling conditions as those for cysteamine described above. Table 1 compares the results for both compounds under these conditions (80% or more remained as  $[^{11}\text{C}]\text{CH}_3\text{I}$ ), although small quantities of N-methylated compounds were observed. The hydroxy group of ethanolamine was not methylated.

### Effect of carrier $\text{CH}_3\text{I}$ on the labeling efficiency of cysteamine by $[^{11}\text{C}]\text{CH}_3\text{I}$

The effects of carrier  $\text{CH}_3\text{I}$  on the labeling efficiency

**Table 1**  $[^{11}\text{C}]$ -methylation of cysteamine and ethanolamine

| Structure                            | Cysteamine | Ethanolamine |
|--------------------------------------|------------|--------------|
| N,N,N-tri-methyl-cysteamine          | n.d.       | 3.5          |
| N,N-di-methyl-cysteamine             | n.d.       | 12.5         |
| $[^{11}\text{C}]\text{CH}_3\text{I}$ | 1.0        | 83.1         |
| S-methyl-cysteamine                  | 98.5       | —            |

Each numerical value represents the mean of 3 trials. Cysteamine:  $\text{H}_2\text{N}-\text{C}_2\text{H}_4-\text{SH}$ , Ethanolamine:  $\text{H}_2\text{N}-\text{C}_2\text{H}_4-\text{OH}$ .

n.d.: not detected.

Labeling condition:  $1 \times 10^{-5}$  mole of each precursor in 0.25 mL of 1 N NaOH and 0.1 mL of acetone (including  $[^{11}\text{C}]\text{CH}_3\text{I}$ ) at 0°C with a reaction time of 5 min.

**Table 2** Effect of methyl iodide carrier and reaction temperature on labeling efficiency of cysteamine by  $[^{11}\text{C}]\text{CH}_3\text{I}$

| $\text{CH}_3\text{I}$                       | No addition |      | 4.26 mg<br>( $3 \times 10^{-5}$ mole) | 14.2 mg<br>( $1 \times 10^{-4}$ mole) |      |
|---|-------------|------|---------------------------------------|---------------------------------------|------|
| temp. (°C)                                  | 0           | 50   | 0                                     | 0                                     | 50   |
| S- $[^{11}\text{C}]$ -methyl-Cy             | 98.5        | 98.0 | 81.6                                  | 35.8                                  | 42.8 |
| S,N,N-tri- $[^{11}\text{C}]$ -methyl-Cy     | n.d.        | n.d. | n.d.                                  | n.d.                                  | 3.8  |
| S,N,N,N-tetra- $[^{11}\text{C}]$ -methyl-Cy | n.d.        | n.d. | n.d.                                  | 1.2                                   | 11.7 |
| $[^{11}\text{C}]\text{CH}_3\text{I}$        | 1.0         | 1.0  | 18.4                                  | 63.2                                  | 41.7 |

Each value represents the mean of 3 trials.

Cy: Cysteamine, n.d.: not detected.

labeling condition: 1.14 mg Cy ( $1 \times 10^{-5}$  mole) in 0.25 mL of 1 N NaOH and 0.1 mL of acetone (including  $\text{CH}_3\text{I}$ ) at reaction time of 5 min.

TLC analysis (Silica gel; developing solvent: chloroform/methanol/ammonia=8/2/1) and HPLC analysis with reverse-phase column; eluting solution: acetonitrile/water/methylamine=49/50/1 at a flow rate of 0.5 mL/min.

**Table 3** S-methylation of mercapto compounds

| Compound                           | Chemical structure           | Reaction temperature (°C) | Non-reacted [ $^{11}\text{C}$ ]CH $_3$ I (%) | Proportion of the product (%) <sup>#</sup> | TLC |
|------------------------------------|------------------------------|---------------------------|--|--|-----|
| 2-mercapto-acetic acid             | HS-CH $_2$ -COOH             | 0                         | 2.8  | >99  | a)  |
|                                    |                              | 50                        | n.d.   | >99  | a)  |
| 3-mercapto-propionic acid          | HS-C $_2$ H $_4$ -COOH       | 50                        | 3.0  | >99  | b)  |
| 2-mercapto-ethylamine (cysteamine) | HS-C $_2$ H $_4$ -NH $_2$    | 0                         | 1.0  | 98.5                                       | c)  |
|                                    |                              | 50                        | 1.0  | 98.0                                       | c)  |
| 2-mercapto-ethanol                 | HS-C $_2$ H $_4$ -OH         | 0                         | n.d.   | 98.2                                       | d)  |
|                                    |                              | 50                        | 4.5  | 94.5                                       | d)  |
| 3-mercapto-3-deoxy-glycerol        | HS-CH $_2$ CH(OH)-CH $_2$ OH | 50                        | n.d.   | >99  | e)  |

<sup>#</sup> The proportion of the desired product was calculated from the peak area in TLC.

\* TLC developing solvent system

a) chloroform/methanol/ammonia=7/3/1

b) chloroform/methanol/ammonia=2/9/1

c) chloroform/methanol/ammonia=8/2/1

d) chloroform/acetone=9/1

e) chloroform/methanol=8/2

n.d.: not detected.

Each value presents the mean of 3 trials.

of cysteamine by [ $^{11}\text{C}$ ]CH $_3$ I were investigated (Table 2). By increasing the amount of CH $_3$ I carrier, the proportion of S-methyl-cysteamine was reduced to 81.6% and 35.8% with  $3 \times 10^{-5}$  and  $1 \times 10^{-4}$  mole of CH $_3$ I respectively, at 0°C. At 50°C, a higher yield of S-methyl-cysteamine (42.8%) was obtained and a lower content of non-reacted [ $^{11}\text{C}$ ]CH $_3$ I remained. The proportions of S,N,N-tri-methyl-cysteamine and S,N,N,N-tetra-methyl-cysteamine were 3.8% and 11.7%, respectively.

#### *S-[ $^{11}\text{C}$ ]-methylation reaction of five thiol compounds*

The five thiol compounds in Table 3 were methylated in a similar manner to that described above with [ $^{11}\text{C}$ ]CH $_3$ I in a basic solution, and the products were assayed by TLC with the developing solvents shown in Table 3. Each compound was selectively S-methylated at both 0 and 50°C, whereas only the latter temperature was employed in 2-mercapto-ethanol. The proportion of the desired compound was lower (94%) at 50°C than at 0°C (98%).

### DISCUSSION

The reactivity and selectivity of [ $^{11}\text{C}$ ]CH $_3$ I to the thiol group, and the effects of other functional groups on S-methylation reaction were evaluated in this study.

Before radioactive methylation reactions were attempted, non-radioactive methylation trials of various thiol compounds were conducted in 5 min

at 0°C without other by-products. This rapid cold reaction was performed to evaluate the reactivity of the radioactive precursor, [ $^{11}\text{C}$ ]CH $_3$ I, with the thiol group of cysteamine. High reactivity of the thiol group with [ $^{11}\text{C}$ ]CH $_3$ I was observed by stirring at 0°C for only 1 min in a basic solution without a catalyst (Fig. 1). Hence, [ $^{11}\text{C}$ ]-methylation of the thiol group required only 1-min stirring with no catalyst. In addition, no N-methylated compound was detected under these conditions. Moreover, the N-methylation reaction did not proceed at 50°C. The difference between the cold synthesis and C-11 reaction was attributed to the varied quantities of the CH $_3$ I carrier. In fact, in the [ $^{11}\text{C}$ ]-methylation reaction, N-methylated compounds were produced by increasing the quantity of the CH $_3$ I carrier (Table 2). Although the structure of ethanolamine is similar to that of cysteamine, the amino group of ethanolamine was methylated under conditions similar to that of cysteamine (Table 1). Consequently, these facts indicate that the reactivity of the thiol group with methyl iodide is much higher than that of the amino group. In addition, results obtained with the other compounds containing hydroxy groups or carboxylic groups showed high selectivity for the thiol group in the [ $^{11}\text{C}$ ]CH $_3$ I methylation.

In conclusion, the usefulness of this reaction between the thiol group and methyl iodide was readily achieved without any heating. Because most biologically active substances have chemically unstable groups in their structures, it is important to reduce

those side reactions in order to produce labeled compounds with a high radiochemical yield and purity. Consequently, the S-methylation reaction reported here can be used to synthesize readily available [ $^{11}\text{C}$ ]-labeled radiopharmaceuticals. Further evaluations of novel drugs by means of the S-methylation reaction are currently in progress.

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