The Synthesis of Tritium-Labeled Methyl Iodide and L-Methionine

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SUMMARY

A procedure for the preparation of methyl iodide-$^3H_3$ of high specific activity starting with tritium gas, its use for the synthesis of tritium-labeled L-methionine-[methyl-$^3H_3$] are described. The tritiated L-methionine can be used as a building block for the preparation of tritium labeled peptide containing terminal L-methionine.

Keywords: Methyl iodide-$^3H_3$, L-Methionine-[methyl-$^3H_3$], L-Methionine-[methyl-$^3H_3$] methyl ester

INTRODUCTION

The methyl functionality is found in a great many biologically active molecules. To have these molecules enriched with three tritium atoms at high specific activity for biological studies is desirable, since methylation using C^3H_3I can often be performed in the last step of the synthetic sequence. A review article has been published in the literature for the preparattion of tritium labeled methyl iodide.[1] The methods are all unattractive for the routine preparation of tritiated methyl iodide because of low yields, large number of steps, low specific activity or large reaction scale. It has been known for many years that carbon-14 labeled methyl iodide can be prepared by the reduction of carbon dioxide to methanol with $LiAlH_4$, followed by conversion to methyl iodide.[2] This process was not applied for tritium labeling because $LiAl^3H_4$ was not available at that time. In 1990, H. Andres et al. published a simple and facile synthesis of $LiAlH_4$.[3] This method provided an easy access to the tritium labeled reducing reagent. Using deuterium as a model, we were able to prepare $LiAl^2H_4$ starting with 0.5 mmol of deuterium gas. Subsequently, methyl iodide-$^2H_3$ was prepared by the reduction of carbon dioxide to methanol-$^2H_3$ followed by treatment with HI. The overall chemical yield based on the

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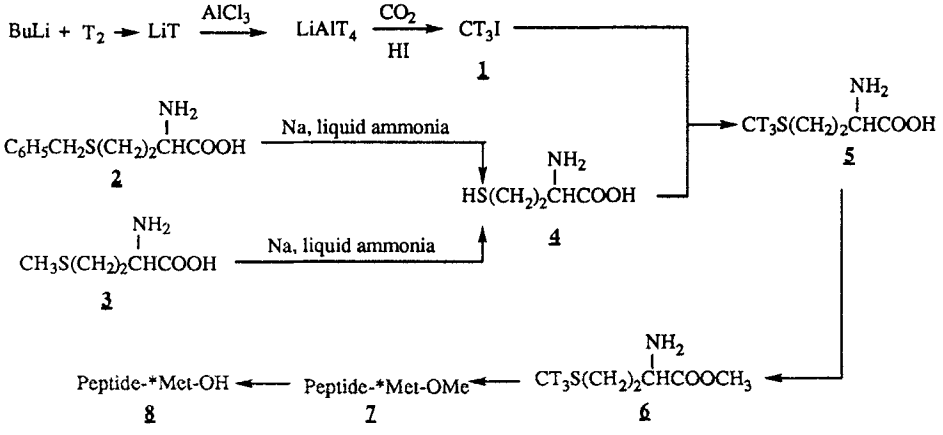
deuterium gas was 8%. Methylation of a tertiary amine, dimethylbenzylamine, to its quaternary salt using this methyl iodide-$^2H_3$ gave a $^2H_3$ overall incorporation of 96% in the resulting salt without dilution of incorporation. Following the same process, C^3H_3I (1) was prepared and successfully utilized for the preparation of tritium labeled L-methionine-[methyl-$^3H_3$] (5) starting with L-methionine (3) as shown in the following scheme.



As reported in the literature,[4,5] methionine may be prepared by the methylation of homocysteine (4) in liquid ammonia using methyl iodide, which would be the labeling reagent. The L-homocysteine could be generated in turn from either L-2-amino-4-(benzylthio)butyric acid (2)[5] or L-methionine (3)[6] by the use of sodium in liquid ammonia. The optical rotation of the final product of a cold run, L-methionine, was taken and the result showed that there was no racemization. We chose L-methionine as the starting material because both cold starting material and the labeled product are the same compound. Therefore, only one amino acid, L-methionine, would be present in the reaction mixture in case of incomplete reaction, and purification would not be required after reaction. However, it is more desirable to start with compound 2 if labeled L-methionine with higher specific activity without dilution is preferred. A model study using C^2H_3I prepared in the same manner as described above showed a $^2H_3$ incorporation of 36% in compound 5 from 3 (analyzed by MS and NMR) when the molar ratio of 1 to 3 used for the reaction was 2.8. Methylation of 5 in concentrated hydrochloric acid and 2,2-dimethoxy propane gave L-methionine-[methyl-$^3H_3$] methyl ester (6). One step condensation of a peptide residue with this reagent followed by hydrolysis and HPLC purification gave a tritiated peptide containing L-methionine as the terminal amino acid. From 20 Ci of tritium gas, we have prepared a total of 24 mCi of a tritiated peptide (8) with a specific activity of 7.9 Ci/mmol and a radiochemical purity of >99%. The specific

activity was significantly lower than the theoretical maximum of 29.7 Ci/mmol for methyl iodide-$^3H_3$ calculated from the dilution of tritium gas by hydrogen (0.33 mmol to 1 mmol). This apparent decrease in specific activity could be due to the inadvertent introduction of cold methionine into the synthesis of compound **8** due to incomplete demethylation of methionine in the synthesis of compound **5**. Based on the 36 % incorporation observed by the model study using larger excess of deuterated methyl iodide-$^2H_3$, the highest specific activity that could be achieved for **8** would be 10.7 Ci/mmol.

In conclusion, this procedure allows the preparation of C^3H_3I with high specific activity using a manageable quantity of tritium gas for a regular radio-synthetic laboratory. Furthermore, L-methionine-[methyl-$^3H_3$] can be used as a building block for the preparation of various tritium labeled peptides containing terminal L-methionine in the molecule.

EXPERIMENTAL

Methyl Iodide-$^3H_3$ (1)

A solution of 0.625 mL (1 mmol) of n-BuLi in hexane (1.6 M) and 0.2 mL (1.32 mmol) of N,N,N',N'-tetramethylethylenediamine (TMEDA) were charged, under nitrogen, into a one piece apparatus consisting of a 10 mL flask with a condenser and a stopcock attached. After addition, this mixture was frozen in a liquid nitrogen bath and then evacuated. To the flask was added 19.8 Ci (0.33 mmol, 60 Ci/mmol) of tritium gas. After stirring at room temperature for 1.5 hours, 0.423 mmol of hydrogen gas was added. This was stirred for 30 minutes at room temperature, and an additional 0.367 mmol of hydrogen gas was added. The highest threotical specific activity of LiT that could be achieved was 9.9 Ci/mmol based on the dilution of tritium gas. The reaction was stirred at room temperature overnight. The solvent and TMEDA were then removed by vacuum transfer at room temperature. The flask was filled with nitrogen and to the residue was added 1.14 mL (0.25 mmol) of a solution containing 585 mg of $AlCl_3$ in 20 mL of anhydrous ether. After stirring at room temperature for 1.5 hours, the mixture was frozen using a liquid nitrogen bath and then evacuated. To the flask was added 3.28 mL (0.132 mmol) of carbon dioxide by vacuum transfer. The reaction mixture was stirred in an ice bath for 60 minutes and then at room temperature for 30 minutes. After the solvent was removed by vacuum transfer, the flask was filled with nitrogen. To this flask was added 0.6 mL (7.96 mmol) of HI (d=1.7). This solution was heated at 80°C for 30 minutes in a closed system. Methyl iodide-$^3H_3$ and excess HI were collected, by vacuum transfer, in a trap cooled in a liquid nitrogen bath. The trap was allowed to warm up to -40°C

and methyl iodide-$^3H_3$ was vacuum transferred into a 10 mL flask cooled in a liquid nitrogen bath. This process was repeated twice more (the last vacuum transfer was carried out into a flask containing calcium hydride), and 15.7 mg (0.11 mmol) of dry methyl iodide-$^3H_3$ was obtained by a final vacuum transfer. The threotical specific activity was 29.7 Ci/mmol and the total activity was 3.267 Ci.

L-Methionine-[Methyl-$^3H_3$] (5)

Into a two neck flask containing 11 mg (0.0738 mmol) of L-methionine was condensed 15 mL of liquid ammonia (the ammonia was passed through a sodium hydroxide drying tower prior to condensing in the flask). The flask was equipped with a cold finger, drying tube containing calcium sulfate (Drierite), and nitrogen bubbler. To this flask, cooled in a dry ice/acetone bath at -80°C, was added 15.5 mg (0.67 mmol) of sodium. The blue color persisted for 30 minutes. To this flask was added 80 mg (1.49 mmol) of ammonium chloride and the reaction mixture was stirred for further 10 minutes at -80°C. Into the flask was vacuum transferred 15.7 mg (0.11 mmol, 3.267 Ci) of methyl iodide-$^3H_3$ (molar ratio of **1** to **3** was 1.49) and the reaction mixture was stirred at -40°C for 30 minutes. After evaporation of the liquid ammonia using a nitrogen stream, the residue was dissolved in 2.1 mL of water. TLC on silica gel eluting with chloroform:methanol:water:acetic acid (12:7:1:0.1) showed a single radioactive product at Rf=0.45. This product was contaminated with some non-radioactive salts, but was used for the next step without purification. The total activity was assayed to be 315 mCi.

L-Methionine-[Methyl-$^3H_3$] Methyl Ester Hydrochloride (6)

Into a 10 mL flask was transferred 2.1 mL of an aqueous solution of L-methionine-[methyl-$^3H_3$] from the previous step and the water was removed by vacuum transfer. To the dried residue was added 1.5 mL of 0.2N HCl / 2.2-dimethoxypropane (prepared by mixing 10 mL of 2.2-dimethoxypropane and 0.15 mL of concentrated hydrochloric acid) and the reaction mixture was stirred at room temperature. The course of the reaction was monitored by TLC on silica gel using the same solvent system as described for the previous step. The Rf of the desired product was 0.9. After 24 hours, the reaction was >90% complete. The mixture was then filtered, the solvent was removed by vacuum transfer and the residue was treated with 10 mL of methanol. This mixture was filtered and the methanol was removed from the filtrate by vacuum transfer. The resultant residue (154 mCi) was used in the next step without further purification. Pure L-methionine-[methyl-$^3H_3$] methyl ester could be obtained by purification on a Waters μBondapak C_{18} analytical column, eluting with 0.05% TFA in water at a flow rate of 2 mL/min (retention time=10 min). The compound was detected by UV at 210 nm.

Biotin-Gly-Leu-Pro-Cys(Farn)-Val-Ile-Met-[Methyl-$^3H_3$]-OMe (7)

A solution containing 20 mg (19.4 μmol) of Biotin-Gly-Leu-Pro-Cys(Farn)-Val-Ile-OH in 1 mL of DMF and 1 mL of methylene chloride was stirred at 0°C, then 10.2 mg (74.93 μmol) of 1-hydroxy-7-azabenzotriazole (HOAt) was added, followed by the addition of 160 μL (92 μmol) of 10% N,N-diisopropylethylamine in DMF. This solution was transferred to the flask containing the crude L-methionine-[methyl-$^3H_3$] methyl ester hydrochloride from the previous step. Stirring was continued at 0°C for a further 5 minutes and 8.2 mg (21.58 μmol) of O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) was added. Stirring was continued at 0°C for one hour and at room temperature for 18 hours. The reaction was analyzed by HPLC on a Waters μBondapak C_{18} column (0.39 x 30 cm), using the following system:

Solvent:	A: 0.05 % TFA in water
	B: 0.05 % TFA in acetonitrile
	Gradient: 40% B to 80% B in 45 minutes
Flow Rate:	2 mL/min
Detection:	UV 210 nm and Radioactivity (^3H, IN/US β-Ram)
Retention time:	19 min

Based on the radiochromatogram, 60 % of the total radioactivity was associated with the desired product. The solvent was removed by vacuum transfer and the residue was transferred to a 15-mL polypropylene tube using 7 mL of methylene chloride. This solution was washed with 2x2 mL of saturated sodium bicarbonate solution, 2x2 mL of 10 % citric acid, 2x2 mL of water, and 2x2 mL of brine. Analysis by HPLC showed only one radioactive compound to be present. The methylene chloride was removed by a gentle nitrogen stream and the resultant residue was used in the next step without further purification.

Biotin-Gly-Leu-Pro-Cys(Farn)-Val-Ile-Met-[Methyl-^3H]-OH (8)

To compound 7 from the previous step, in the polypropylene tube, was added 3 mL of acetonitrile, 2.5 mL of water, and 0.8 mL of 0.1 N sodium hydroxide. The resultant mixture was stirred at room temperature for 5 hours, then stored in the refrigerator at 4°C overnight. Analytical HPLC of the reaction mixture using the same system as described for the methyl ester (7) indicated that >90% of 7 had been hydrolyzed (retention time for the product was 15 min. 34 sec.). The mixture was acidified to pH 4-6 with glacial acetic acid, as indicated by a litmus paper test. After removal of the majority of the acetonitrile by a gentle nitrogen stream, the resultant, concentrated solution was stored in the refrigerator overnight. The solid that precipitated out of the solution was collected by centrifugation, then dissolved

in 3.3 mL of water/acetonitrile solution (1:1) and purified by HPLC on a Waters μBondapak C_{18} analytical column in several injections using the following system:

Solvent:	A: 0.05 % TFA in water
	B: 0.05 % TFA in acetonitrile
	Gradient: 40% B to 60% B in 60 minutes
Flow Rate:	2 mL/min
Detection:	UV 210 nm and Radioactivity (^3H, Ramona flow through HPLC detector)
Retention time:	33 min

The product fractions were collected in a 50-mL polypropylene tube and was concentrated to a total of 14.4 mL by a gentle nitrogen stream. The total radioactivity was determined to be 24 mCi by liquid scintillation counting. The total weight was 3.52 mg as determined by HPLC and UV analysis. The specific activity was then calculated to be 7.9 Ci/mmol. The radiochemical purity was determined to be 99.2 % by HPLC on a Waters μBondapak C_{18} analytical column. The sample was lyophilized to dryness and was stored in a -80°C freezer under argon.

REFERENCES

1. Liebman A.A., Malarek D.H., Serico L., Choudhry S. C., and Muccino R.R. - Trans. Amer. Nuc. Soc. 55: 40 (1987)
2. Murray A. III and Williams D.L.- Organic Syntheses with Isotopes, Interscience Publishers, Inc., New York, pp. 861, 899 (1958)
3. Andres H., Morimoto H., and Williams P. G. - J. Chem. Soc., Chem. Commun. 627 (1990).
4. Murray A. III and Williams D.L.,- Organic Syntheses with Isotopes, Interscience Publishers, Inc., New York, pp. 1302-1304 (1958).
5. Biochemical Preparations Volume 5, D. Shemin, Ed. Wiley, New York, pp. 91-92 (1957)
6. K. Hamacher, - J. Labeled Compounds and Radiopharmaceuticals 27: 343 (1989)