Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVI, No. 4

Synthesis of Carbon-11 Labeled Methylcarbamates from [^{11}C]-Methylchloroformate

Hayden T. Ravert*, William B. Mathews, John L. Musachio, and Robert F. Dannals

Divisions of Nuclear Medicine and Radiation Health Sciences
The Johns Hopkins Medical Institutions
615 North Wolfe Street, Baltimore, MD 21205-2179

Summary

[^{11}C]-Methylchloroformate, a novel [^{11}C]-acylating agent, was generated *in situ* from [^{11}C]-methanol and phosgene. To explore the utility of [^{11}C]-methylchloroformate, this agent was reacted with several amines to yield their corresponding [^{11}C]-labeled methylcarbamates. The average synthesis (including purification and formulation) required approximately 23 minutes from end of bombardment. The average specific activity was calculated to be approx. 607 mCi/μmole at end of synthesis with an average radiochemical yield of 6%, decay corrected to starting [^{11}C]-methanol. Preliminary results reveal that [^{11}C]-methylchloroformate is a useful general reagent for the preparation of [^{11}C]-methyl carbamates of both primary and secondary amines.

Key Words: Methylcarbamate, carbon-11, positron emission tomography, antihistamine

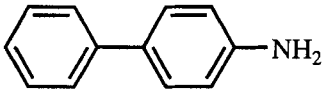
Introduction

The synthesis of radioligands labeled with carbon-11 for use in positron emission tomography (PET) has become a standard practice in many PET research laboratories. A number of carbon-11 labeled reactive species have been synthesized to introduce carbon-11 into a tracer (1). One of the most common molecules is [^{11}C]-methyl iodide. Others include [^{11}C]-labeled phosgene, cyanide, formaldehyde, acid chlorides, nitromethane, methyl triflate and alkyl iodides.

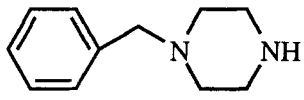
* Author for correspondence.

CCC 0362–4803/95/040365–07
©1995 by John Wiley & Sons, Ltd.
Received 11 October, 1994
Revised 15 November, 1994

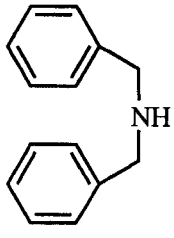
Previously, [^{11}C]-labeled ethylchloroformate, prepared from [^{11}C]-labeled phosgene and unlabeled ethanol, was reacted with primary amine derivatives of estradiol and hexesterol (2, 3). However, the radiosynthesis afforded the product in low yield (10 - 30% end of bombardment) and specific activity (1 - 20 mCi/μmole). No reactions using labeled methylchloroformate have been reported. We have chosen to investigate an alternate route for the synthesis of alkyl carbamates; specifically, the synthesis of [^{11}C]-labeled methylchloroformate from [^{11}C]-methanol and its reaction with amines to form labeled methylcarbamates. Described here is the reaction of [^{11}C]-methylchloroformate with three structurally simple amines (Figure 1) to determine conditions for the formation of labeled methylcarbamates. A more complex amine, SCH 34117, (Figure 1) was reacted under the same conditions to prepare the methylcarbamyl analog of SCH 29851 (loratadine, an ethyl carbamate), a potent antihistamine.



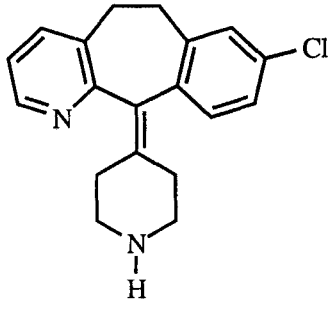
Biphenylamine



Benzylpiperazine



Dibenzylamine



SCH 34117

Figure 1: Structures of amines.

Results and Discussion

The production of carbon-11 labeled methylchloroformate and subsequent reaction with an amine is illustrated in Scheme 1. The initial reduction of $^{11}CO_2$ with lithium aluminum hydride and removal of THF solvent proceeded smoothly.

$$^{11}CO_2 \xrightarrow[THF]{LiAlH_4} {}^{11}CH_3O^- \xrightarrow[distill]{H_2O\ /\ glycol\ ether} {}^{11}CH_3OH + Cl\text{-}\overset{\overset{O}{\|}}{C}\text{-}Cl \rightarrow {}^{11}CH_3OCOCl$$

$$^{11}CH_3OCOCl + HNR_2 \xrightarrow{TEA} {}^{11}CH_3OCONR_2$$

Scheme 1.

However, initial attempts to extract or directly distill [^{11}C]-methanol proved cumbersome. Extraction of the radioactivity, after the addition of aqueous 1 N HCl, into methylene chloride yielded only 4% extracted activity. Direct distillation from 1 N HCl yielded approximately 1% of the radioactivity in a chilled vessel of methylene chloride.

A recent literature procedure described the distillation of [^{11}C]-methanol from 5% water/diethyleneglycol monobutylether solution (4). This procedure proved reliable and consistent. Varying the quantity of water present in the water/diethyleneglycol monobutylether solution from 1 to 5% did not affect the yield of [^{11}C]-methanol produced. However, short path distillation allowed small quantities of the water/diethyleneglycol monobutylether to be transferred into the reaction vessel. In addition, a large portion of the radioactivity was trapped in residual liquid in the transfer line and needle. Placing a small P_2O_5 trap in the line did reduce the liquid transfer but the trap also retained a large portion of the [^{11}C]-methanol (approximately 60%). The best transfer of radioactivity was observed when a small column (8 mm ID x 50 mm L) packed loosely with glass wool was installed vertically between the reduction/hydrolysis vessel and the collection vessel. The column impeded solvent droplets while allowing the [^{11}C]-methanol into the reaction vial. Greater than 90% transfer of radioactivity was observed.

After collection of the [^{11}C]-methanol in chilled methylene chloride, [^{11}C]-methylchloroformate was generated *in situ* following the addition of a solution of phosgene and allowing the reaction vessel to warm to room temperature. If the phosgene was added to the vial prior to collection of the radioactivity, the yield of [^{11}C]-methylchloroformate was reduced. A possible reason for the reduced yield with prior addition may be the loss of phosgene due to its volatility during the [^{11}C]-methanol transfer. The yield of [^{11}C]-methylchloroformate after 3 minutes at room temperature was 51% (n = 6) based on the radiochromatograph peak areas. Increasing the reaction time or temperature had no significant affect on the yield. The remaining radioactivity (presumably [^{11}C]-methanol) was observed as a peak at the HPLC column void volume.

At first triethylamine (TEA) was added to the reaction vial, prior to the addition of the amine, to react with any hydrogen chloride formed during the subsequent acylation reaction. In a few of the reactions a precipitate formed indicating the formation of triethylamine hydrochloride. In these cases the hydrogen chloride was most likely generated by the reaction of phosgene with a trace amount of water. Due to an excess of TEA in the reaction solution, the acylation reaction did proceed to completion when precipitate formed. Later attempts showed this stepwise addition to be unnecessary.

HPLC data indicated that the reaction of [^{11}C]-methylchloroformate with all four amines was complete in one minute at room temperature. The radiochromatograms show the complete disappearance of [^{11}C]-methylchloroformate and the formation of corresponding radiolabeled products in approximately 25% yield based on total radioactivity in the chromatogram. The remaining radioactivity was observed as a peak at the HPLC column void volume. Although phosgene was present in a two fold or more excess compared to the amines, it did not appear to interfere with the reaction of [^{11}C]-methylchloroformate with the amine substrates. After addition of HPLC solvent and evaporation of methylene chloride with warming, the mixture was injected onto a preparative reverse phase HPLC column. The [^{11}C]-labeled carbamates were collected and the solvent evaporated *in vacuo*. After formulation in saline, an aliquot was removed to determine purity and specific activity. Isolated radiochemical yields for the amines, decay corrected to starting [^{11}C]-methanol, varied between 1 and 15% (average of 6%) with an average synthesis time of approximately 23 minutes from end of bombardment. The average radiochemical yield for methylcarbamyl SCH 34117 (n = 3) was 8.2% (from [^{11}C]-methanol) with an average synthesis time of 17.3 minutes and an average specific activity of 607 mCi/μmole. The chemical purity of the isolated [^{11}C]-methylcarbamyl SCH 34117 was greater than 95%. The preparative purification for the reactions involving the simple amines was not optimized for complete resolution of the precursor and radiolabeled product; for these compounds the chemical purities were between 50 - 75%.

The synthesis of [^{11}C]-methylchloroformate from [^{11}C]-carbon dioxide and subsequent acylation reaction with purification proved facile in these initial attempts. The yield and synthesis time were appropriate for synthetic carbon-11 chemistry. The acylation of primary and secondary amines with [^{11}C]-methylchloroformate may prove a useful method of synthesizing new tracers for use with PET.

Experimental

1-Benzylpiperazine, dibenzylamine and diphenylamine were obtained from the Aldrich Chemical Company and used without further purification. SCH 34117 was provided as a gift by the Schering-Plough Research Institute (Dr. Allen Barnett). 20% phosgene in toluene was obtained from the Fluka Chemical Company. All reagents were ACS or HPLC purity. Proton NMR spectra were obtained on a Bruker AMX300 spectrometer in the Johns Hopkins University Biophysics Laboratory. Melting points were obtained on a Melt-Temp Laboratory Device and are uncorrected. High performance liquid chromatographic analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line fixed wavelength (254 nm) detector, and a single two inch NaI crystal radioactive detector. HPLC chromatograms were recorded by a Rainin Dynamax dual channel

control/interface module connected to a Macintosh computer with appropriate program software (Dynamax version 1.2). HPLC semipreparative purification of three of the compounds were completed on an Prep Nova-Pak HR C_{18} 6 μ column (7.8 x 300 mm) using a mobile phase of 60% acetonitrile/40% water (0.1 M ammonium formate) at a flow rate of 7 mL/min. The fourth, carbomethoxy-1-benzylpiperazine, was purified on the same column with a mobile phase of 30% acetonitrile/70% water (0.1 M ammonium formate) at the same flow rate. Chemical and radiochemical purity were determined using an Nova-Pak 4 μ C_{18} HPLC column (3.9 x 150 mm) with the same mobile phase as in the semipreparative HPLC at a flow rate of 3 mL/min. [^{11}C]-Methylchloroformate has an analytical k' of 0.5 and 1.7 using a mobile phase of 60% acetonitrile/40% water (0.1 M ammonium formate) and 30% acetonitrile/70% water (0.1 M ammonium formate) respectively. A dose calibrator (Capintec CRC-12R) was used for all radioactivity measurements.

General synthesis of methylcarbamates

A solution of 1.5 mmol of methylchloroformate in 10 mL of methylene chloride was added dropwise to a stirred solution of 1.5 mmol of secondary amine and 1.5 mmol of triethylamine in 20 mL methylene chloride under argon positive pressure. After addition of the chloroformate was completed, the solution was stirred for 15 minutes and the solvent was evaporated under vacuum. The solid was redissolved in 10 mL of methylene chloride and washed with 2 x 10 mL of water. The methylene chloride layer was dried with sodium sulfate and evaporated under vacuum.

The compounds were characterized by elemental analysis. The following describes physical characterization and elemental analysis (calculated and found) for: N-carbomethoxydibenzylamine ($C_{16}H_{17}NO_2$) colorless oil, Cal'd: C, 75.27; H, 6.71; N, 5.49, Found: C, 76.09; H, 6.82; N, 5.51; N-carbomethoxy-1-benzyl-piperazine ($C_{13}H_{18}N_2O_2 \cdot 0.25\ H_2O$) mp 35 - 38° C, Cal'd: C: C, 65.38; H, 7.81; N, 11.73, Found: C, 65.32; H, 7.64; N,.11.73; N-carbomethoxy-4-aminobiphenyl ($C_{14}H_{13}NO_2$ colorless oil, Cal'd: C, 73.99, H, 5.76, N, 6.17, Found: C, 73.56, H, 5.85, N, 5.93; and N-carbomethoxy-SCH 34117 ($C_{21}H_{21}ClN_2O_2$) mp 149 - 150° C, Cal'd: C, 68.38; H, 5.74; N, 7.60, Found: C, 68.46; H, 5.79; N, 7.52.

The compounds were also characterized by proton NMR. ^{1}H NMR ($CDCl_3$) N-carbomethoxydibenzylamine: δ: 3.78 (s, 3H), 4.40 (d, 4H), 7.12 (m, 10H); N-carbomethoxy-1-benzylpiperazine: δ: 2.50 (m, 4H), 3.62 (m, 4H), 3.65 (s, 2H), 3.82 (s, 3H), 7.47 (m, 5H); N-carbomethoxy-4-aminobiphenyl: δ: 3.84 (s, 3H), 7.52 (m, 9H), 9.11 (bs, 1H); and N-carbomethoxy-SCH 34117: δ: 2.36 (m, 4H), 2.75 (m, 2H), 3.09 (m, 2H), 3.29 (m, 2H), 3.66 (s, 3H), 3.77 (m, 2H), 7.02 (m, 4H), 7.39 (d, 1H), 8.33 (d, 1H).

The compounds were characterized by analytical and preparative HPLC under the conditions described above and the capacity factors (k') for the precursors and products determined (see table below).

	k'	
Compound	analytical	preparative
Dibenzylamine	1.3	1.9
N-Carbomethoxy-dibenzylamine	2.2	2.9
1-Benzylpiperazine	2.5	3.9
N-Carbomethoxy-1-benzylpiperazine	4.7	4.8
4-Aminobiphenyl	1.6	2.1
N-Carbomethoxy-4-aminobiphenyl	4.0	3.9
SCH 34117	0.3	0.1
N-Carbomethoxy-SCH 34117	2.8	1.9

General radiosynthesis and purification of [^{11}C]-methylcarbamates.

[^{11}C]-Methanol was synthesized by a modification of a previously described method (4). Briefly cyclotron produced [^{11}C]-carbon dioxide was collected in an evacuated stainless steel loop cooled with liquid nitrogen. Following the collection of radiolabeled gas, the loop was warmed to room temperature and the carbon dioxide was transferred with nitrogen gas into a conical reaction vessel containing 150 µL of lithium aluminum hydride in THF (35 mg in 4 mL) and 300 µL of THF. After trapping of [^{11}C]-carbon dioxide was completed, the solution was evaporated to dryness using a heat gun with continued nitrogen gas flow. A solution of 1% water in diethyleneglycol monobutylether (300 µL) was added. Upon heating (approximately 150° C) and with continued gas flow at approximately 30 - 40 mL/min, [^{11}C]-methanol was transferred to a small conical reaction vessel containing 100 µL methylene chloride chilled in a dry ice/ethanol bath. After the radioactivity reached a plateau, the solution was removed from the cold bath and 8 µL of 20% phosgene in toluene was added to the reaction vessel. The reaction mixture was kept at room temperature for 3 minutes.

To the solution of [^{11}C]-methylchloroformate, generated *in situ* as described above, was added the appropriate amine (1 - 2 mg) and 15 µL of triethylamine in 100 µL of methylene chloride. The reaction mixture was kept at room temperature for 1 minute after which preparative HPLC solvent (200 µL) was added to the vial. Argon was bubbled through the solution at room temperature to remove the methylene chloride. After removal of methylene chloride, the solution was injected onto the preparative column and the product with the appropriate retention time was collected in a rotary evaporator under vacuum. Following evaporation of the HPLC mobile phase at 80° C, sterile saline (7 mL) was added to the flask containing the

product and the final product solution was passed through a 0.2 μ filter (Gelman Acrodisc) into a sterile evacuated vial. A 100 μL aliquot was removed for the determination of radiochemical purity and specific activity.

Acknowledgments

The authors wish to thank Mr. Robert C. Smoot, C.N.M.T., for his help in cyclotron operation and radiochemical synthesis. This work was supported in part by the U.S.P.H.S. grant numbers NS-15080, CA-32845, and CA-09199 (WBM).

References

1. Fowler J. S. and Wolf A. P., *Positron Emitter-Labeled Compounds: Priorities and Problems* (Raven Press, New York, 1986).

2. Ali H., Rousseau J., Diksic M., and van Lier J. E. - Int J Rad Appl Instrum B 19: 175 (1992).

3. Ouellet R., Rousseau J., Brasseur N., van Lier J. E., Diksic M., and Westera G. - J Med Chem 27: 509 (1984).

4. Holschbach M., and Schüller M. - Appl Radiat Isot 44: 779 (1993).