

Research Article

Methylation of the thiophene ring using Carbon-11-labelled methyl iodide: formation of 3-[¹¹C]methylthiophene

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Summary

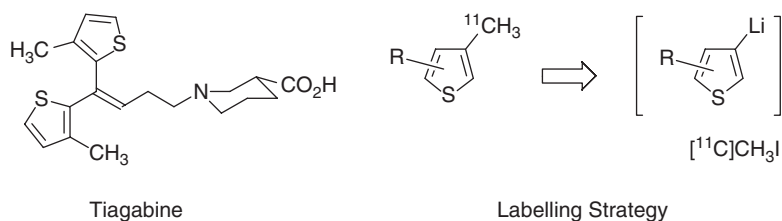
This paper describes the radiosynthesis of 3-[¹¹C]methylthiophene, chosen as a model reaction for the preparation of heteroaromatic methylthienyl compounds. Labelling was performed from the corresponding lithiothiophene derivative and [¹¹C]methyl iodide as the alkylating agent in THF at –78°C. The conditions used were the following: (1) trapping for 2–3 min at –78°C of the [¹¹C]methyl iodide in the THF solution containing the freshly prepared 3-lithiothiophene; (2) Hydrolysis of the reaction mixture by adding 0.5 ml of the HPLC mobile phase and (3) HPLC purification. 3-[¹¹C]Methylthiophene ([¹¹C]-**1**) was collected in high yield as the unique peak of the HPLC radiochromatogram. Non-reacted [¹¹C]methyl iodide was not present. Typically, 50–60 mCi (1.85–2.22 GBq) of 3-[¹¹C]methylthiophene ([¹¹C]-**1**) were obtained within 20 min of radiosynthesis (including HPLC purification) with specific radioactivities ranging from 0.6 to 1.0 Ci/μmol (22.2–37.0 GBq/μmol) starting from 180 to 200 mCi (6.66–7.40 GBq) of [¹¹C]CO₂ (10 μA, 10 min (6000 μC) irradiation). Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: methylation; methyl iodide; thiophene; carbon-11; positron emission tomography; PET

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Introduction

In the course of our PET CNS programs, we were confronted with the challenging radiosynthesis of a suggested potent radioligand for the imaging of the GABA uptake system, namely tiagabine ((R)-1-[4,4-bis(3-methyl-2thienyl)-3-butenyl]-3-piperidinecarboxylic acid, NNC 05-0328).^{1,2}



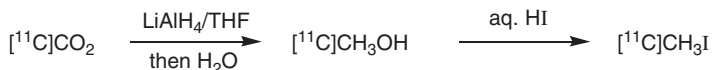
This nipecotic acid derivative possesses in its chemical structure two methylthienyl aromatic rings, which we envisaged to label with carbon-11 at the methyl substituent. Preparations of compounds bearing these rings usually include the use of reagents which already possess the desired methylthienyl moiety. However, examples of direct methylation at the 3-position of the thiophene ring have also been described in the literature. 3-Methylthiophene derivatives were obtained using either methyl iodide³⁻⁶ or dimethyl sulfate⁷⁻⁹. In all cases, the corresponding 3thienyl lithium derivatives were the key-intermediates. They were generated in situ from the 3-bromothiophene derivatives³⁻⁹.

Based on these examples, carbon-11 (a 20.4 min half-life, cyclotron-produced radioisotope) labelling of this heteroaromatic system could be envisaged from the corresponding lithiothiophene derivative, and [¹¹C]methyl iodide as the alkylating agent. In view of the complexity of the Tiagabine molecule, we decided to investigate first the feasibility of these heteroaromatic methylations with [¹¹C]methyl iodide on a simple model: the formation of 3-[¹¹C]methylthiophene. This paper describes the preparation of 3-[¹¹C]methylthiophene from carbon-11 labelled methyl iodide.

Results and discussion

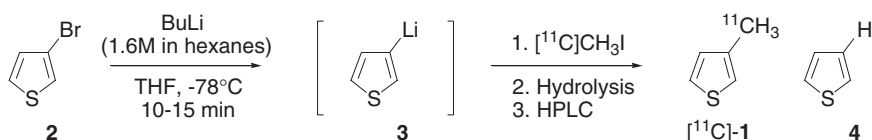
[¹¹C]Methyl iodide was prepared from [¹¹C]carbon dioxide using the well-known two step, one pot protocol, consisting of the trapping of

[^{11}C]carbon dioxide and conversion into [^{11}C]methanol (LiAlH_4) followed by iodination using aqueous HI giving [^{11}C]methyl iodide.¹⁰



On average, about 650 mCi (24.1 GBq) of [^{11}C]CH₃I is routinely obtained in our laboratory in 7–8 min after the end of the bombardment (EOB) in 70% decay-corrected yield, based on starting [^{11}C]carbon dioxide.

Reaction of 3-bromothiophene (**2**, 10.7 μmol) with butyl lithium solution in hexanes at -78°C (19.2 μmol , 1.8 equivalents) in THF for 10–15 min cleanly gave the 3-lithiothiophene (**3**). Formation of lithio-derivative **3** was monitored at this stage by analysing an aliquot of the reaction mixture with HPLC. The predominant signal detected with the UV-spectrophotometer was observed for thiophene (**4**), resulting from the hydrolysis of the *in situ*-formed lithio-derivative **3** and only a residual signal could be observed for the non-reacted starting material 3-bromothiophene (**2**). Using less butyl lithium (1.0 to 1.6 equivalents), the lithiation reaction did not go to completion and considerable amounts of 3-bromothiophene (**2**) were still observed relative to thiophene (**4**). In all further experiments, 1.8 equivalents of butyl lithium relative to 3-bromothiophene (**2**) were used.



The lithio-derivative **3** was then reacted with [^{11}C]methyl iodide in THF at -78°C . The conditions used were the following: (1) trapping for 2–3 min at -78°C of the [^{11}C]methyl iodide in the THF solution containing the freshly prepared 3-lithiothiophene (**3**); (2) Hydrolysis of the reaction mixture by adding 0.5 ml of the HPLC mobile phase and (3) HPLC purification. 3-[^{11}C]Methylthiophene ([^{11}C]-**1**) was collected as the unique peak of the HPLC radioactive chromatogram. Non-reacted [^{11}C]methyl iodide was usually not present and 3-[^{11}C]methylthiophene ([^{11}C]-**1**) was obtained in high yield.

Typically, 50–60 mCi (1.85–2.22 GBq) of 3- ^{11}C methylthiophene (^{11}C -**1**) were obtained within 20 min of radiosynthesis (including HPLC purification) with specific radioactivities ranging from 0.6 to 1.0 Ci/ μmol (22.2–37.0 GBq/ μmol) starting from 180 to 200 mCi (6.66–7.40 GBq) of ^{11}C CO₂ (10 μA , 10 min (6000 μC) irradiation). This corresponds to a 75–90% decay-corrected yield of 3- ^{11}C methylthiophene (^{11}C -**1**) based on starting ^{11}C methyl iodide.

The absence of ^{11}C methyl iodide in these optimized conditions (1.8 equivalents of butyl lithium relative to 3-bromothiophene (**2**)) is on the one hand due to the high yields of conversion into 3- ^{11}C methylthiophene (^{11}C -**1**). On the other hand, conversion of ^{11}C methyl iodide by residual butyl lithium to ^{11}C methyl lithium, giving ^{11}C methane on hydrolysis, can explain the absence of ^{11}C methyl iodide in the final reaction mixture when only a 75% yield of formation of 3- ^{11}C methylthiophene (^{11}C -**1**) was observed. This conversion is well characterized and used in the carbon-11 chemistry field for the preparation of high amounts of ^{11}C methyl lithium.¹¹

When the 3-lithiothiophene (**3**) derivative was prepared using only a slight excess of butyl lithium (12.8 μmol , 1.2 equivalents for example) using similar conditions to those described above (10.7 μmol of 3-bromothiophene **2**, butyl lithium solution in hexane at -78°C in THF for 10–15 min), yields of formation of 3- ^{11}C methylthiophene (^{11}C -**1**) was systematically lower and high amounts of non-reacted ^{11}C methyl iodide were observed. This shows that less than 10 μmol of 3-lithiothiophene (**3**) are not sufficient to drive the methylation reaction to its maximum yield, even though 1–3 μmol of 3-lithiothiophene (**3**) are still a considerable excess of reagent over ^{11}C methyl iodide. This also shows that no residual butyl lithium is present in the final reaction mixture since if this were the case ^{11}C methyl iodide would have been transformed into ^{11}C methyl lithium, giving volatile ^{11}C methane on hydrolysis.

Experimental

General: Chemicals were purchased from standard commercial sources (Aldrich, Fluka or Sigma France) and were used without further purification unless stated otherwise. HPLC: Equipment: Waters or Shimadzu systems. HPLC A: Waters systems equipped with a 510 pump, 440 UV detector or 481/486 UV-multiwavelength detectors; column: semipreparative C-18 Zorbax® SB, Hewlett Packard

(250 × 9.4 mm); porosity: 5 μm; conditions: isocratic elution with: MeCN/water/THF: 40/55/5; flow rate: 7.0 ml/min; temperature: RT; UV detection at λ: 230 nm; R_t : thiophene: 5.5 min, 3-methylthiophene: 8.5 min, 3-bromothiophene: 9.7 min; HPLC B: Waters systems equipped with a 510 pump, 440 UV detector or 481/486 UV-multiwavelength detectors; column: semipreparative C-18 Zorbax® SB, Hewlett Packard (250 × 9.4 mm); porosity: 5 μm; conditions: isocratic elution with: MeCN/water/THF: 50/45/5; flow rate: 7.0 ml/min; temperature: RT; UV detection at λ: 230 nm; R_t : thiophene: 4.5 min, 3-methylthiophene: 6.6 min, 3-bromothiophene: 7.3 min; HPLC C: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: semipreparative C-18 Zorbax® SB, Hewlett Packard (250 × 9.4 mm); porosity: 5 μm; conditions: isocratic elution with: MeCN/water/THF: 50/45/5; flow rate: 7.0 ml/min; temperature: RT; UV detection at λ: 230 nm; R_t : 3-methylthiophene: 6.6 min. Radiosyntheses were performed in a 5-cm-lead shielded confinement.

Preparation of [^{11}C]CO₂: [^{11}C]CO₂ was produced by irradiation of an ultrapure N60 Air Liquide N₂ target with a 20 MeV proton beam (30 μA) via the $^{14}\text{N}[\text{p},\alpha]^{11}\text{C}$ nuclear reaction on a CGR-MeV 520 cyclotron. At the end of the bombardment, the target contents were transferred to the 5-cm-lead shielded hot cell dedicated to the radiosynthesis of the tracer and passed firstly through a glass P₂O₅-guard (70 mm length, 3 mm internal diameter) in order to remove moisture. [^{11}C]CO₂ was then separated from the target gas by trapping in an empty stainless-steel coil (1500 mm length, 0.51 mm internal diameter), cooled at -186°C using liquid argon. On average, about 1.20 Ci or 44.40 GBq (EOB) of [^{11}C]CO₂ is routinely obtained in our laboratory for a 30 μA, 30 min (54000 μC) irradiation.

Preparation of [^{11}C]CH₃I: [^{11}C]CO₂ was released from the trap by simply raising the stainless-steel coil to room temperature, swept away by a flow of nitrogen gas (40 ml/min) and trapped at -10°C (EtOH-ice bath) into 55 μl of THF containing 5 μl of 1.0 M THF solution of lithium aluminium hydride. Concentration to dryness (evaporation of solvent at 165°C using a stream of nitrogen) followed by hydrolysis (100 μl of deionized water) of the formed aluminium complex afforded [^{11}C]CH₃OH, which was distilled, using a flow of nitrogen gas, into 1 ml of an aqueous 57% HI solution (heating block at 165°C). The [^{11}C]CH₃I

thus synthesized was continuously swept away by the flow of nitrogen gas and passed through a combined 1/1 (v:v) soda lime/P₂O₅-guard (35 mm length each, 3 mm internal diameter). About 650 mCi (24.1 GBq) of [¹¹C]CH₃I is routinely obtained in our laboratory in 7–8 min after EOB in 70% decay-corrected yield, based on starting [¹¹C]CO₂.

Preparation of 3-lithiothiophene (3): 12 μl of a 1.6 M butyl lithium solution in hexane were added to 10 μl of a cooled (−78°C, acetone/dry-ice bath) 1.067 M solution of 3-bromothiophene (**2**, 10.7 μmol) in THF (100 μl of 3-bromothiophene (**2**) diluted with 900 μl of dry THF) in a 2 ml reaction vessel. The mixture was left without stirring at this temperature for 10–15 min. At this stage, a 1 μl aliquot of the solution was diluted with water (10 μl) and analysed by HPLC (HPLC A or B). Formation of the 3-lithiothiophene **3** was monitored by calculating from the HPLC chromatogram the ratio of the thiophene peak (predominant signal, resulting from the hydrolysis of the lithio derivative) over the 3-bromothiophene peak (residual signal of non-reacted starting material).

Preparation of 3-[¹¹C]methylthiophene ([¹¹C]-1): [¹¹C]CH₃I, carried by a flow of nitrogen gas, was trapped (bubbling through) at −78°C (acetone/dry-ice bath) in the THF solution containing the freshly prepared 3thienyl lithium. Trapping of [¹¹C]CH₃I was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), the crude product was taken up with 0.5 ml of the HPLC mobile phase and an aliquot was injected onto the column (HPLC A or HPLC B).

Quality control of 3-[¹¹C]methylthiophene ([¹¹C]-1): As demonstrated by HPLC analysis (HPLC C), the radiolabelled product was found to be >99% radiochemically pure and also co-eluted with a sample of authentic 3-methylthiophene (**1**). The purified 3-[¹¹C] methylthiophene ([¹¹C]-**1**) was shown to be free of non-radioactive precursor. Specific radioactivity was calculated from three consecutive HPLC analyses and determined as follows: The area of the UV absorbance peak corresponding to the radio-labelled product was measured (integrated) on the HPLC chromatogram and compared to a standard curve relating mass to UV absorbance (HPLC C).

Conclusion

This paper described the first example of a carbon-11 radiosynthesis of a methylthienyl compound. Preparation of 3-[¹¹C]methylthiophene was chosen as a model reaction: Labelling of this heteroaromatic system was performed from the corresponding lithiothiophene derivative and [¹¹C]methyl iodide as the alkylating agent. Typically, 50–60 mCi (1.85–2.22 GBq) of 3-[¹¹C]methylthiophene ([¹¹C]-**1**) were obtained within 20 min of radiosynthesis (including HPLC purification) with specific radioactivities ranging from 0.6 to 1.0 Ci/μmol (22.2–37.0 GBq/μmol) starting from 180 to 200 mCi (6.66–7.40 GBq) of [¹¹C]CO₂ (10 μA, 10 min (6000 μC) irradiation).

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