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Direct Incorporation of $[^{11}\text{C}]\text{CO}_2$ into asymmetric $[^{11}\text{C}]\text{carbonates}$

Abdul Karim Haji Dheere*, Salvatore Bongarzone*, Dinah Shakir and Antony Gee

Department of Chemistry and Biology, School of Biomedical Engineering and Imaging Sciences, King's College London, King's Health Partners, St. Thomas' Hospital, London, UK.

* These authors contributed equally to this work.

Correspondence should be addressed to Antony Gee; antony.gee@kcl.ac.uk

Abstract

A novel carbon-11 radiolabelling methodology for the synthesis of the dialkylcarbonate functional group has been developed. The method uses cyclotron-produced short-lived $[^{11}\text{C}]\text{CO}_2$ (half-life 20.4 min) directly from the cyclotron target in a one-pot synthesis. Alcohol in the presence of base trapped $[^{11}\text{C}]\text{CO}_2$ efficiently forming an $[^{11}\text{C}]\text{alkylcarbonate}$ intermediate that subsequently reacted with an alkylchloride producing the di-substituted $[^{11}\text{C}]\text{carbonate}$ (34% radiochemical yield, determined by radio-HPLC) in 5 minutes from the end of $[^{11}\text{C}]\text{CO}_2$ cyclotron delivery.

Introduction

Positron emission tomography (PET) is an imaging technique able to detect and monitor specific target proteins in vivo.[1] The use of PET imaging has advanced in the last few decades to become a valuable tool in clinical diagnostics, medical research and drug discovery.[2] PET relies on the use of tracer amounts of imaging probes (radiotracers). The administration of radiotracers allows biochemical process to be imaged and quantified in vivo without manifestation of pharmacological or toxicological effects.[3]

Carbon-11 ($^{11}\text{C}$) is one of the most common radionuclides used for the synthesis of PET radiotracers. The short half-life of $^{11}\text{C}$ (20.4 min) makes it an attractive radionuclide as it enables the collection of a sufficient amount of PET data while keeping the subject radiation dose and exposure time to minimum. Furthermore it allows orthologus substitution with
carbon-12 in biologically active molecules with no alteration of the parent molecule’s physicochemical and pharmacological properties. Carbon-11 is commonly produced in the form of $[^{11}\text{C}]\text{carbon dioxide} ([^{11}\text{C}]\text{CO}_2).$ $[^{11}\text{C}]\text{CO}_2$ is usually converted into more reactive secondary precursors such as $[^{11}\text{C}]\text{methyl iodide} ([^{11}\text{C}]\text{CH}_3\text{I}),$ $[^{11}\text{C}]\text{carbon monoxide} ([^{11}\text{C}]\text{CO}),$ and $[^{11}\text{C}]\text{phosgene} ([^{11}\text{C}]\text{COCl}_2).$ As these multistep conversion processes are time consuming, the use of $[^{11}\text{C}]\text{CO}_2$ for directly radiolabelling functional groups is highly attractive. $[^{11}\text{C}]\text{CO}_2$ is a weak electrophile with an affinity for electron-donating reagents such as amines and organometallics. However, due to the thermodynamic and kinetic properties of $[^{11}\text{C}]\text{CO}_2,$ it has high activation energy which requires the use of highly reactive reagents, temperatures, pressures, or the presence of a catalyst. Nevertheless, the primary synthon, $[^{11}\text{C}]\text{CO}_2,$ has been deployed successfully for the synthesis of $^{11}\text{C}$-compounds that contain carbonyl groups such as $[^{11}\text{C}]\text{carbamates},$ amide, and $[^{11}\text{C}]\text{ureas.}$ However, the radiolabelling of the carbonyl group of carbonates from $[^{11}\text{C}]\text{CO}_2$ has not yet been established. To date, the synthesis of $[^{11}\text{C}]\text{carbonates}$ has relied on the use of $[^{11}\text{C}]\text{COCl}_2$ which is produced from a multistep process starting from cyclotron-produced $[^{11}\text{C}]\text{CH}_4,$ conversion to $[^{11}\text{C}]\text{CCl}_4$ and then to $[^{11}\text{C}]\text{COCl}_2.$ Although this $^{11}\text{C}$-carbonate reaction is rapid and efficient, routine production of $[^{11}\text{C}]\text{COCl}_2$ requires a multistep syntheses and specialized equipment, thereby restricting its widespread use.

As the carbonate functional group is found in prodrug compounds as well as being an intermediate in organic synthesis, we aimed to develop a simple and robust radiolabelling methodology that uses $[^{11}\text{C}]\text{CO}_2$ for the synthesis of $[^{11}\text{C}]\text{carbonates.}$ Here we present a rapid, one-pot radiosynthetic strategy using $[^{11}\text{C}]\text{CO}_2$ directly from the cyclotron, avoiding the need for specialized equipment and multistep syntheses.

**Materials and Methods**

All purchased chemicals were used without further purification. Chemicals were purchased in highest available purity from Sigma-Aldrich and Alfa Aesar and used as received (> 99 % purity). All solvents were purchased as anhydrous in highest available purity (> 99.8 % purity) from Sigma-Aldrich.

$[^{11}\text{C}]\text{CO}_2$ was produced by a Siemens RDS112 cyclotron (St Thomas’ Hospital, London, United Kingdom) via the $^{14}\text{N}(p,\alpha)^{11}\text{C}$ nuclear reaction. Typical irradiation times for exploratory work was 1 minute, 10 µA, bombardment typically yielding ca. 300 MBq $[^{11}\text{C}]\text{CO}_2$ at end of
cyclotron bombardment. Radiolabelling reactions were performed in a 1.5 mL screw top vial with a “V” internal shape. HPLC analysis was performed on an Agilent 2060 Infinity HPLC system with a variable wavelength detector (254 nm was used as default wavelength). An Agilent Eclipse XDB-C18 reverse-phase column (4.6 x 150 mm, 5 µm) was used at a flow rate of 1 mL/min and H₂O/MeOH (HPLC grade solvents with 0.1 % TFA) gradient elution (flow rate: 1 mL/min, 0-2 min: 5 % MeOH, 2-11 min: 5 to 95 % MeOH linear gradient, 11-13 min: 90 % MeOH, 13-14 min: 90 % to 5 % MeOH linear gradient, and 14-15 min: 5 % MeOH). The RCY was estimated by radio-HPLC and defined as the area under the ¹¹C-product peak expressed as a percentage of the total ¹¹C labelled peak areas observed in the chromatogram. Molar radioactivity was calculated from analytical HPLC sample of 25 µL. A calibration curve of known mass quantity versus HPLC peak area (254 nm) was used to calculate the mass concentration of the 25 µL radiolabelled compound. The identity of the radiolabelled compound peak was confirmed by HPLC co-injection of a nonradioactive reference compound and yielded a single peak.
Results and Discussion

![Chemical Reaction](image)

**Figure 1.** Method by Salvatore et al. [7] for the synthesis of carbonates using non-radioactive CO₂.

As a starting point we selected the method developed by Salvatore et al. [7] (Figure 1) for the synthesis of carbonates. The established method used non-radioactive CO₂, an alcohol derivative and benzylchloride (BzCl) in the presence of Cs₂CO₃, TBAI in DMF to produce the corresponding carbonate derivative efficiently. By substituting CO₂ with [¹¹C]CO₂ and applying the same reaction conditions, the synthesis of di-substituted [¹¹C]carbonates was investigated.
Table 1 Optimisation of $[^{11}\text{C}]\text{I}$ synthesis.

<table>
<thead>
<tr>
<th>Entry$^{[a]}$</th>
<th>Base</th>
<th>Trapping efficiency (%)</th>
<th>Temperature ($^\circ\text{C}$)</th>
<th>Solvent</th>
<th>RCY (%)$^{[b]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cs$_2$CO$_3$</td>
<td>95.2</td>
<td>25</td>
<td>DMF</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>Cs$_2$SO$_4$</td>
<td>1.5</td>
<td>25</td>
<td>DMF</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>CsI</td>
<td>4.3</td>
<td>25</td>
<td>DMF</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>CsF</td>
<td>33.5</td>
<td>25</td>
<td>DMF</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>K$_2$CO$_3$</td>
<td>10</td>
<td>25</td>
<td>DMF</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>CaCO$_3$</td>
<td>0</td>
<td>25</td>
<td>DMF</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Cs$_2$CO$_3$</td>
<td>20</td>
<td>25</td>
<td>CH$_3$CN</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Cs$_2$CO$_3$</td>
<td>65</td>
<td>25</td>
<td>DMSO</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Cs$_2$CO$_3$</td>
<td>&gt;95%</td>
<td>65</td>
<td>DMF</td>
<td>33</td>
</tr>
<tr>
<td>11$^{[c]}$</td>
<td>Cs$_2$CO$_3$</td>
<td>&gt;95%</td>
<td>100</td>
<td>DMF</td>
<td>82, 74</td>
</tr>
</tbody>
</table>

$^{[a]}$ Reaction conditions: Isopropanol (22 µmol), Cs$_2$CO$_3$ (66 µmol), TBAI (66 µmol) and organohalide (66 µmol) in 500 µL DMF, 10 mins from end of delivery (EOD) (n=1). $^{[b]}$ The non-isolated radiochemical yield determined by radio-HPLC analysis of the crude product. $^{[c]}$ n=2

$[^{11}\text{C}]\text{CO}_2$ was trapped in isopropyl alcohol in the presence of Cs$_2$CO$_3$, forming an $[^{11}\text{C}]$isopropylcarbonate intermediate that subsequently reacted with BzCl to produce $[^{11}\text{C}]$benzyl isopropyl carbonate ($[^{11}\text{C}]\text{I}$) in a moderate radiochemical yield (RCY)$^{[13]}$ of 24% (Table 1, entry 1). Interestingly, almost all the cyclotron-produced $[^{11}\text{C}]\text{CO}_2$ was trapped within the reaction mixture at room temperature (> 95%); any unreacted radioactive $[^{11}\text{C}]\text{CO}_2$ was immobilized on an ascarite trap connected to the vial vent needle.$^{[14]}$
In an attempt to increase the RCY, Cs$_2$CO$_3$ was replaced with Cs$_2$SO$_4$ (Table 1, entry 2). The trapping efficiency of [$^{11}$C]CO$_2$ dropped significantly from 95.2% to 1.5%. Since Cs$_2$CO$_3$ contributed towards the trapping of [$^{11}$C]CO$_2$ efficiently, we investigated whether the Cs$^+$ or the CO$_3^{2-}$ ion was responsible for the high [$^{11}$C]CO$_2$ trapping efficiency. Of a number of caesium bases explored (Table 1, entries 3-5), CsI and CsF trapped only minute amounts of [$^{11}$C]CO$_2$ (4% and 34%, respectively), indicating that the basicity of the reaction mixture had a major effect on trapping efficiency. These results can be explained by the ability of a strong base to deprotonate the alcohol present in the reaction mixture enabling it to react with [$^{11}$C]CO$_2$ to form a $^{11}$C radiolabelled intermediate. The importance of CO$_3^{2-}$ was then explored by comparing Cs$_2$CO$_3$ with other carbonate bases (K$_2$CO$_3$ and CaCO$_3$, Table 1, entries 6 and 7). The trapping efficiencies were extremely low for both reagents. High trapping in the reaction mixture with Cs$_2$CO$_3$ is therefore most likely due to its superior solubility in organic solvents.

**Table 2** Optimisation of [$^{11}$C]I synthesis using alternative bases.

<table>
<thead>
<tr>
<th>Entry$^a$</th>
<th>Base (eq)</th>
<th>TBAI (eq)</th>
<th>Temp (°C)</th>
<th>RCY (%)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^c$</td>
<td>DBU (3)</td>
<td>3</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>2$^c$</td>
<td>DBU (3)</td>
<td>-</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>NaH (1)</td>
<td>1</td>
<td>100</td>
<td>26</td>
</tr>
<tr>
<td>4$^d$</td>
<td>NaH (1)</td>
<td>1</td>
<td>60</td>
<td>31±2</td>
</tr>
<tr>
<td>5$^e$</td>
<td>NaH (0.5)</td>
<td>1</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>NaH (2)</td>
<td>1</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>7$^e$</td>
<td>NaH (0.5)</td>
<td>-</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>NaH (1)</td>
<td>3</td>
<td>60</td>
<td>7</td>
</tr>
</tbody>
</table>

$^a$ Isopropanol (1 equiv., 22 µmol), BzCl (3 equiv.), TBAI (1-3 equiv.) and base (1-3 equiv.) in 500 µL DMF reaction time 5 mins from EOD.

$^b$ The non-isolated radiochemical yield determined by radio-HPLC analysis of the crude product.

$^c$ Reaction time of 10 mins from EOD.

$^d$ n=3
In a further attempt to increase the RCY of $[^{11}C]I$, a number of aprotic solvents were screened (CH$_3$CN and DMSO, Table 1, entries 8 and 9). However, these solvents did not produce $[^{11}C]I$ and the trapping efficiency was poor (20% and 65%, respectively). Reaction dependency on temperature was subsequently examined. The RCY of $[^{11}C]I$ improved from 24% to 33% by increasing the reaction temperature from 25 °C to 65 °C (Table 1, entry 10). Increasing the temperature to 100 °C promoted the product formation and resulted in the highest observed RCY (82%, Table 1, entry 11). This might be rationalised by an increase in Cs$_2$CO$_3$ solubility at higher temperatures. However, due the presence of Cs$_2$CO$_3$ as a reagent, low molar activities ($A_m$) were observed. The low $A_m$ (2 GBq/μmol in this case) is likely due to release of non-radioactive CO$_2$ from Cs$_2$CO$_3$. CO$_3^{2-}$ deprotonates the alcohol to form HCO$_3^-$, which at high temperature has the potential to decompose releasing non-radioactive CO$_2$ causing isotopic dilution and low $A_m$ of the $[^{11}C]$CO$_2$. We therefore focused on improving $A_m$ by substituting Cs$_2$CO$_3$ with an alternative base.

1,8-diazabicyclo[5.4.0]undecene (DBU) is a basic amine that has been shown to retain $[^{11}C]$CO$_2$ in organic solutions.[9] Replacing Cs$_2$CO$_3$ with DBU (Table 2, entry 1) resulted in $[^{11}C]I$ formation, but with low RCY (6%). The low RCY could be due to DBU being unable to deprotonate isopropyl alcohol efficiently. We opted for a stronger base, NaH, which was able to deprotonate the isopropyl alcohol. Using a ratio of 1:1 NaH:isopropanol (equiv.) at 100 °C, $[^{11}C]I$ was obtained with a RCY of 26% (Table 2, entry 3). Decreasing the temperature from 100 °C to 60 °C slightly improved the RCY (31%, Table 2, entry 4).[15] Decreasing the ratio of NaH:isopropanol (from 1:1 to 0.5:1) reduced the RCY further to 18% (Table 2, entries 5). Increasing the ratio NaH:isopropanol 2:1 did not produce the target product (Table 2, entry 6). Increasing the amount of TBAI to 3 equiv. or removing it completely also did not improve the RCY (Table 2, entries 7 and 8).

**Conclusions**

In conclusion, we have developed a radiolabelling methodology for the synthesis of $[^{11}C]$carbonates using $[^{11}C]$CO$_2$ directly from the cyclotron. The carbonate $[^{11}C]I$ was synthesized by bubbling $[^{11}C]$CO$_2$ into a solution containing alkylchloride, alcohol and a base in DMF. The choice of the base was critical for maximising the RCY and $A_m$. The first protocol uses Cs$_2$CO$_3$ and produces the target $^{11}$C radiolabelled product in a high RCY and low $A_m$. The second strategy, which uses NaH, produced $[^{11}C]I$ in high $A_m$ and moderate RCY. These
methodologies are a simple and practical alternative to $^{11}$C-phosgene for the synthesis of $^{11}$C-carbonates. $^{11}$C-phosgene synthesis is technically challenging to implement and requires the use of specialist equipment. The developed strategies described here use readily available labware and converts $[^{11}C]CO_2$ directly to $[^{11}C]$carbonates in rapid synthesis times.

**Data Availability**

The necessary data used to support the findings of this study are included within the article. Any additional data that may be of interest to readers are available from the corresponding author upon request.

**Conflicts of Interest**

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

**Funding Statement**

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**References**


13. The RCY is the non-isolated radiochemical yield determined by radio-HPLC analysis of the crude product.

14. The trapping efficiency is the amount of radioactivity trapped in the reaction vial as a percentage of the overall radioactivity produced by the cyclotron.

15. $[^{11}C]1$ was produced with a molar activity (A_m) of 10 – 20 GBq/umol. This is because short cyclotron bombardments (1 minute) and low beam currents (5 – 10 µA) were used (0.3 GBq). In clinical productions at our facility, cyclotron bombardment times of 50 minutes and beam currents of 30 µA are...
used to produce higher amounts of radioactivity (typically 60 GBq). It is therefore estimated that this would increase the $A_m$ to $> 50$ GBq/µmol at end of synthesis.