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Direct ^{11}C -Methylation of Amines from $[^{11}\text{C}]\text{CO}_2$ for the Synthesis of PET Radiotracers.

François Liger,^[b] Ture Eijsbouts,^{[a],[b]} Florence Cadarossanesaib,^[b] Christian Tourvieille,^[b] Didier Le Bars,^{[a],[b]} Thierry Billard*^{[a],[b]}

Abstract: ^{11}C -Methylation ($t_{1/2} = 20.4$ min) is a main labeling strategy in the development of PET radiotracers. A straight radiomethylation of amines by use of cyclotron-produced $^{11}\text{CO}_2$ was developed to obtain various radiolabeled compounds with good radiochemical yields. This strategy constitutes a new approach to simplify the radiolabeling processes and transpositions onto automatic synthesizers. This method was applied to the syntheses of radiolabeled drugs and a PET radiotracer used in Alzheimer's disease studies.

Introduction

PET (positron emission tomography) imaging is a nuclear medicine technology increasingly used not only in routine clinical diagnosis^[1] but also in biomedical research and drug development.^[2] These growing applications of PET imaging require the development of more and more various radiotracers. However, the short-lived positron-emitters impose specific synthetic methods, easily transferable onto automatic synthesizers.^[1c, 3]

In general, [^{18}F] labeled molecules are preferred for production and use in routine clinical imaging due to the higher half-life of fluorine-18, as illustrated by the recent development of various methods for radiofluorination.^[4] Nevertheless, despite the ^{11}C short half-life ($t_{1/2} = 20.4$ min), [^{11}C] labeled molecules still represent valuable targets for research studies. Indeed, easy-to-introduce carbon-substituents are present in a large majority of drugs and bioactive compounds. Consequently, a direct radiolabeled equivalent of molecule is easier to envisage.

The radioisotope carbon-11 is usually produced from a cyclotron by the $^{14}\text{N}(p,\alpha)^{11}\text{C}$ nuclear reaction in the form of either of two precursors: $^{11}\text{CH}_4$ or $^{11}\text{CO}_2$. Direct use of these two compounds is quite limited and they are, usually, converted into more reactive species. Some direct synthetic applications of

$^{11}\text{CO}_2$ have been previously described^[5] to obtain [^{11}C] labeled carboxylic acids by reaction with Grignard and organolithium reagents^[3a, 6] or, more recently, by cross-coupling reactions with boronic acids.^[7] The syntheses of [^{11}C] labeled ureas and carbamates have been also described.^[8]

Methylation reactions of heteroatoms, in particular nitrogen, constitute one of the more classic approaches for ^{11}C -labeling of molecules,^[3a, 6] as illustrated by the radiosynthesis of the β -amyloid imaging radioligand [^{11}C]PIB.^[9] These radiomethylations require preliminary transformation of $^{11}\text{CO}_2$ into very reactive species, $^{11}\text{CH}_3\text{I}$ or $^{11}\text{CH}_3\text{OTf}$.^[3a, 6, 10] These reagents are often used under basic conditions in a strictly anhydrous environment. Such conditions can require additional protection and deprotection steps of incompatible functional groups that substantially prolong and complicate the radiolabeling process. Furthermore, from a technical point of view the simplest radiosynthesis, with a minimum of steps, will lead to an easier automation.^[10-11] Consequently, a direct radiomethylation of amines using $^{11}\text{CO}_2$, generated from cyclotron, appears very attractive. Some previous works have been described,^[12] however, these strategies require a multi-steps procedure: $^{11}\text{CO}_2$ is first fixed with silylated amines (preliminary prepared) to give O-silyl carbamates, which are subsequently reduced in situ by LiAlH_4 (a harsh reducer). Therefore, a more direct and soft procedure could be beneficial.

Results and Discussion

Recently, some groups have described the application of CO_2 as a C1-building block for catalytic methylation of amines.^[13] Such elegant works appeared in perfect adequacy with our effort to develop a simpler and more direct way to perform ^{11}C -methylation of amines. This approach would eliminate the preliminary time-consuming, and sometimes sources of failure during the automatic radiolabeling, steps of preparation of reactive methylating reagents. We have focused our interest onto the strategy developed by Cantat et al. using standard and simple catalytic system (ZnCl_2 , IPr), without use of additional hydrogen gas.^[13b] As within this article, N-methyl aniline (**1a**) has been selected as model substrate (Table 1).

With a similar catalytic system, in the same solvent and at the same temperature than the optimal described conditions, only 3% of expected methylation was observed in 20 min (entry 1). Such disappointing results can be rationalized by the short time of reaction, imposed by ^{11}C half-life, compared to the 20 hours required in original publication. To increase the reaction kinetic, a solvent with similar properties and polarity but with a higher boiling point was selected to increase reaction temperature. Thus, in diglyme, at 150°C , a satisfactory radiochemical yield of 40% has been obtained (entry 2).

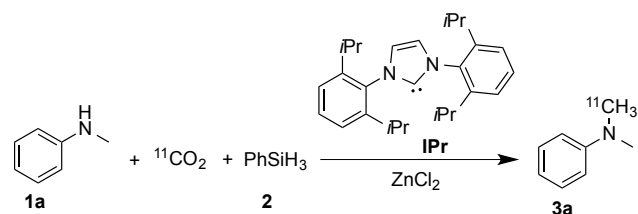
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Because of the small molar amount of $^{11}\text{CO}_2$ generated by cyclotron (e.g. 1 Ci, i.e. 37 GBq, correspond to 108 pmol), the precursor (**1a**) quantity was decreased to match standard amount (around 1-2 mg) used in classic radiolabeling without any change in yield (entry 3). It should be noticed that, for practical reasons (weighing), the same amount of catalyst was preserved, bringing the catalyst amount to stoichiometry (related to **1a**). With a shortened reaction time of 10 min, the radiochemical yield was halved (entry 4), even with a doubled catalyst amount (entry 5). The presence of both ZnCl_2 and **IPr** is proving to be essential (entries 6-8). Preliminary complex formation $[\text{ZnCl}_2;\text{IPr}]^{[14]}$ appears to be deleterious for radiolabeling, in contrast with the work of Cantat et al. (entry 9).^[13b] In order to increase $^{11}\text{CO}_2$ concentration in solution to potentially accelerate the reaction, TMEDA (N,N,N',N'-tetramethylethylenediamine) as CO_2 trap was added, as described by Pike et al.^[7] However, no reaction was then observed: TMEDA certainly competes with **1a** to catch CO_2 (entry 10). Finally, microwave irradiation appeared to be also detrimental for the reaction; the increased molecular agitation contributes to break the amine- CO_2 interaction, which seems to be essential to initiate the methylation (entry 11). Furthermore, Dyson et al. have described similar methylation in metal-free conditions with another carbene (**NHC**, 1,3-bis(2,4,6-trimethylphenyl)-1,3-dihydro-2H-imidazol-2-ylidene).^[13e] Unfortunately, in our conditions, no expected radiolabeling was observed (entry 12).

Moreover, the trapping efficiency of cyclotron-produced $^{11}\text{CO}_2$ was measured using the optimal conditions (Table 1, entry 3) at a trapping flow rate of 10 mL/min. Untrapped $^{11}\text{CO}_2$ was confined within an ascarite[®] cartridge attached to the reactor outlet. Up to 80% of the delivered $^{11}\text{CO}_2$ was kept inside the reactor at the end of entrapment (trapping time = 60s). More precisely, the trapping efficiency depends on the amines basicity: 80% with **1d**, 70% with **1a** and 65% with **1e**.

Table 1. Direct ^{11}C -methylation of **1a** with $^{11}\text{CO}_2$.^[a]



Entry	[1a] (M)	Solvent	T (°C)	t (min)	RCY ^[b] (%)
1	0.231	THF	80	20	3
2	0.231	Diglyme	150	20	40
3	0.023	Diglyme	150	20	43
4	0.023	Diglyme	150	10	23
5 ^[c]	0.023	Diglyme	150	10	27
6 ^[d]	0.023	Diglyme	150	20	0
7 ^[e]	0.023	Diglyme	150	20	0
8 ^[f]	0.023	Diglyme	150	20	3
9 ^[g]	0.023	Diglyme	150	20	6
10 ^[h]	0.023	Diglyme	150	20	0
11 ^[i]	0.023	Diglyme	150	5	4
12 ^[j]	0.023	Diglyme	150	20	0

[a] Conditions: Solvent (400 μL), ZnCl_2 (1,3 mg), **IPr** (3,5 mg), PhSiH_3 (23 μL).

[b] Radiochemical yields (RCY) are estimated from trapped $^{11}\text{CO}_2$ within the reactor and are decay-corrected from the end of $^{11}\text{CO}_2$ trapping inside reactor.

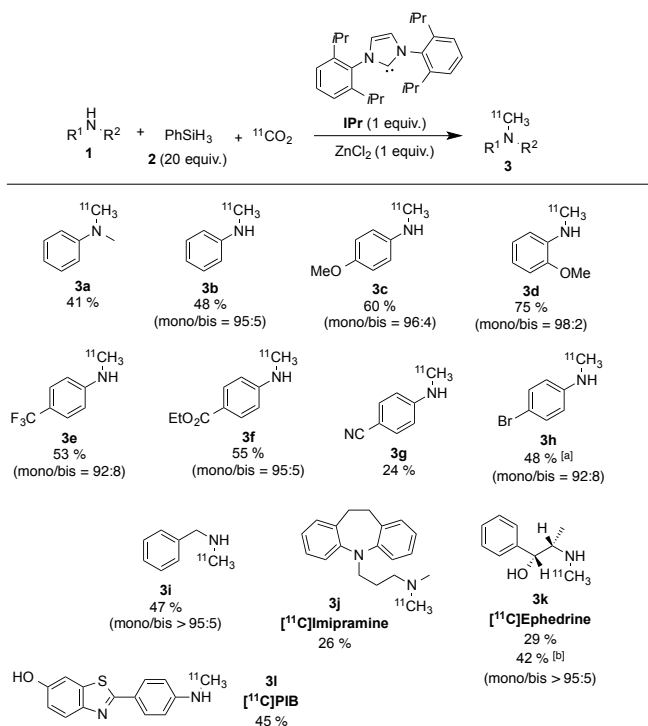
[c] ZnCl_2 (3,5 mg), **IPr** (7 mg). [d] Without ZnCl_2 and **IPr**. [e] Without **IPr**. [f]

Without ZnCl_2 . [g] With preformed complex $[\text{IPr};\text{ZnCl}_2]$ (6 mg). [h] With addition

of TMEDA (70 μL). [i] Under microwave irradiation (140 W). [j] Without ZnCl_2 ,

with **NHC** (2 mg) instead of **IPr** and Ph_2SiH_2 (23 μL) instead of PhSiH_3 .

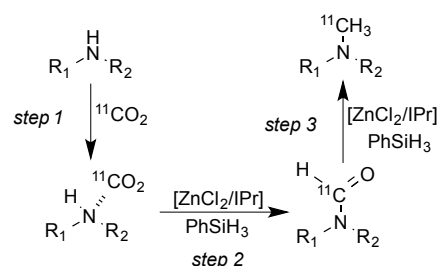
With the optimal reaction conditions in hand (Table 1, entry 3), the scope of this new radiolabeling strategy was investigated using various amines (scheme 1).



Scheme 1. Direct ^{11}C -methylation of amines with $^{11}\text{CO}_2$. RCY values are mean ($n=3$). They are estimated from trapped $^{11}\text{CO}_2$ within the reactor and are decay-corrected from the end of $^{11}\text{CO}_2$ trapping inside reactor. [a] ratio **3h/3b** = 60:40. [b] with 2 equiv. of **1h** vs ZnCl_2 .

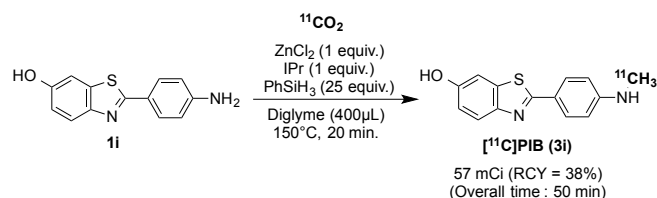
These ^{11}C -methylations gave generally satisfactory radiochemical yields with aromatic or aliphatic amines. Primary amines are selectively mono-methylated and, generally, only a small amount of bis-methylation was observed. With anilines, the presence of electron-withdrawing or electron-donor substituents seems to have no relevant influence (**3b-3h**). In accordance with the supposed mechanism (see scheme 2), electron-withdrawing groups should favor the first step of CO_2 trapping by amine whereas electron-donor substituents should more favor the second and third reduction steps. This reaction is also compatible with ester function (**3f**). In the case of cyano group, a more moderate yield is observed, maybe due to partial chelation of ZnCl_2 by the nitrogen atom partially quenching the reaction. Starting from *p*-bromoaniline (**1h**), a satisfactory yield of expected product was obtained, despite the formation of **3b**, arising from the partial reduction of the C-Br bond. This radiolabeling appears also to be chemoselective since in case of amino-alcohol, only N-methylation is observed (**3k**). However, to achieve similar radiochemical yields compared to other amines, an excess of amino-alcohol (vs ZnCl_2) was required. This could be explained by chelate formation between amino-alcohol and zinc which could deactivate the nitrogen atom and thus would disfavor the CO_2 trapping. Finally, this methodology could be applied to the synthesis of radiolabeled drugs, such as ephedrine (sympathomimetic amine, **3k**), imipramine (tricyclic antidepressant, **3j**) and the β -amyloid radiotracer [^{11}C]PIB (Alzheimer disease diagnostic, **3l**).

From a mechanistic point of view, Cantat et al. have proposed the over reduction of an intermediate formamide (Scheme 2, steps 2-3).^[13b] Furthermore, radiomethylation by reduction of a formamide has been previously reported.^[15] In our conditions the CO_2 -amine interaction appeared to be determining. Indeed, an increase of molecular agitation by micro-wave^[16] disfavored the reaction by breaking this interaction. In addition, starting from imipramine hydrochloride (**3g**), no labeling was observed due to the impossible CO_2 -amine interaction. During the radiomethylation of **1a**, the corresponding labeled formamide, could be identified by HPLC. These observations led us to proposed the following pathway (Scheme 2): 1) formation of CO_2 -amine complex; 2) 1st reduction in formamide; 3) 2nd reduction in expected methyl amine.



Scheme 2. Hypothesized mechanism for the ^{11}C -methylation with $^{11}\text{CO}_2$.

This direct ^{11}C -labeling strategy was applied to effective production of the β -amyloid radiotracer [^{11}C]PIB^[17] (**3l**) with HPLC purification and final formulation step (Scheme 3). [^{11}C]PIB was produced in sufficient radioactive amount (57 mCi – RCY = 38%), with a high radiochemical and chemical purity. Such result is very encouraging and demonstrates the possibility to extrapolate this new strategy to a “productive scale” of radiotracers. Specific radioactivity remains however weak ($\text{SA} = 15 \text{ GBq} \cdot \mu\text{mol}^{-1}$), certainly due to excess of environmental CO_2 which dilutes $^{11}\text{CO}_2$. Nevertheless, in a classical production of [^{11}C]PIB ([^{11}C]methyl triflate method) with the same synthesizer and a similar production of $^{11}\text{CO}_2$ by the same cyclotron, [^{11}C]PIB is obtained with a similar radiochemical yield of 45% and a specific activity of around $50 \text{ GBq} \cdot \mu\text{mol}^{-1}$.



Scheme 3. Production of β -amyloid radiotracer [^{11}C]PIB. RCY value is estimated from trapped $^{11}\text{CO}_2$ within the reactor and are decay-corrected from the end of $^{11}\text{CO}_2$ trapping inside reactor

Conclusions

In conclusion, we have brought the proof of concepts of direct and selective amine ^{11}C -methylation by direct use of cyclotron-produced $^{11}\text{CO}_2$. Even if some improvements are still required, these preliminary data should open the way for new developments of direct and simple radiolabeling methods.

Experimental Section

Synthesis of [^{11}C]PIB (**3i**). Cyclotron-produced $^{11}\text{CO}_2$, trapped (953 mCi) on a column of molecular sieves (4 Å), was released by purging the heated column (350°C) with He gas and bubbled, through Teflon lines, into the reactor containing ZnCl_2 (1.3mg), **IPr** (3.5 mg), **1i** (1.7 mg) in 400 μL of diglyme and PhSiH_3 (23 μL) cooled at 0°C ($^{11}\text{CO}_2$ traps: 711 mCi). Teflon lines were removed when radioactivity content reached a maximum and the reacting mixture was heated at 150°C for 20 min. After cooling, 2.2 mL of HPLC mobile phase were added to the mixture and the resulting solution was purified by HPLC on a Waters Symmetry-Prep C18 column (7 μm , 7.8 x 300 mm) at a flow rate of 4 mL/min ($\text{H}_2\text{O}/\text{MeCN}$ 60:40 v/v). [^{11}C]PIB fraction (t_{R} = 11 min) was collected, diluted in 40 mL of water and formulated by SPE. After rinsing with 10 mL of water, the purified product was released from the Sep-Pak (Waters Plus tC18) with 1 mL of ethanol and 2 mL of water in a sterile vial (57 mCi).

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Keywords: alkylation • carbon-11 • positron emission tomography • radiochemistry • isotopic labeling

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