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Direct [^{11}C]Methylation of Amines from [^{11}C]CO_2 for the Synthesis of PET Radiotracers

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[^{11}C]Methylation ($t_{1/2}$ = 20.4 min) is a main labeling strategy for the development of PET (positron emission tomography) radiotracers. A straight radiomethylation of amines with cyclotron-produced $^{11}CO_2$ has been developed to obtain various radiolabeled compounds with good radiochemical yields. This strategy is a new approach to simplifying radiolabeling processes and transpositions onto automatic synthesizers and has been applied to the synthesis of radiolabeled drugs and a PET radiotracer used in the study of Alzheimer's disease.

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 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 longer half-life of fluorine-18, as illustrated by the recent development of various methods for radiofluorination.[4] Nevertheless, despite the short half-life of carbon-11 ($t_{1/2}$ = 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 a molecule is easier to envisage.

The radioisotope carbon-11 is usually produced in a cyclotron by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in the form of either of two precursors, $^{11}CH_4$ or $^{11}CO_2$. The direct use of these two compounds is quite limited and they are usually converted into more reactive species. Some direct synthetic applications of $^{11}CO_2$ have previously been described[5] for the production of ^{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 synthesis of ^{11}C-labeled ureas and carbamates have also been described.[8]

Methylation reactions of heteroatoms, in particular nitrogen, is one of the more classic approaches used for the ^{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 $^{11}CO_2$ into very reactive species, $^{11}CH_3I$ or $^{11}CH_3OTf$.[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, the direct radiomethylation of amines using $^{11}CO_2$, generated in a cyclotron, appears very attractive. Some previous work in this area has been described,[12] however, these strategies require a multistep procedure: $^{11}CO_2$ is first fixed with silylated amines (previously prepared) to give *O*-silyl carbamates, which are subsequently reduced in situ by $LiAlH_4$ (a harsh reducer). Therefore, a more direct and milder procedure could be beneficial.

Results and Discussion

Recently, some groups described the application of CO_2 as a C_1 building block for the catalytic methylation of amines.[13] Such elegant work appeared in perfect adequacy with our effort to develop a simpler and more direct approach to the [^{11}C]methylation of amines. This approach would eliminate the preliminary time-consuming steps,

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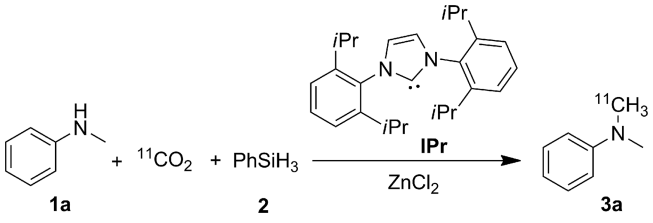
which are also sometimes the source of failure during the automatic radiolabeling, required for the preparation of reactive methylating reagents. We focused our attention on the strategy developed by Cantat and co-workers who employed a standard and simple catalytic system {$ZnCl_2$, IPr [IPr = 1,3-bis(2,6-diisopropylphenyl)-1,3-dihydro-2*H*-imidazol-2-ylidene]} without the use of additional hydrogen gas.[13b] As in their article, *N*-methylaniline (**1a**) was selected as a model substrate (Table 1).

Table 1. Direct [11C]methylation of **1a** with $^{11}CO_2$.[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

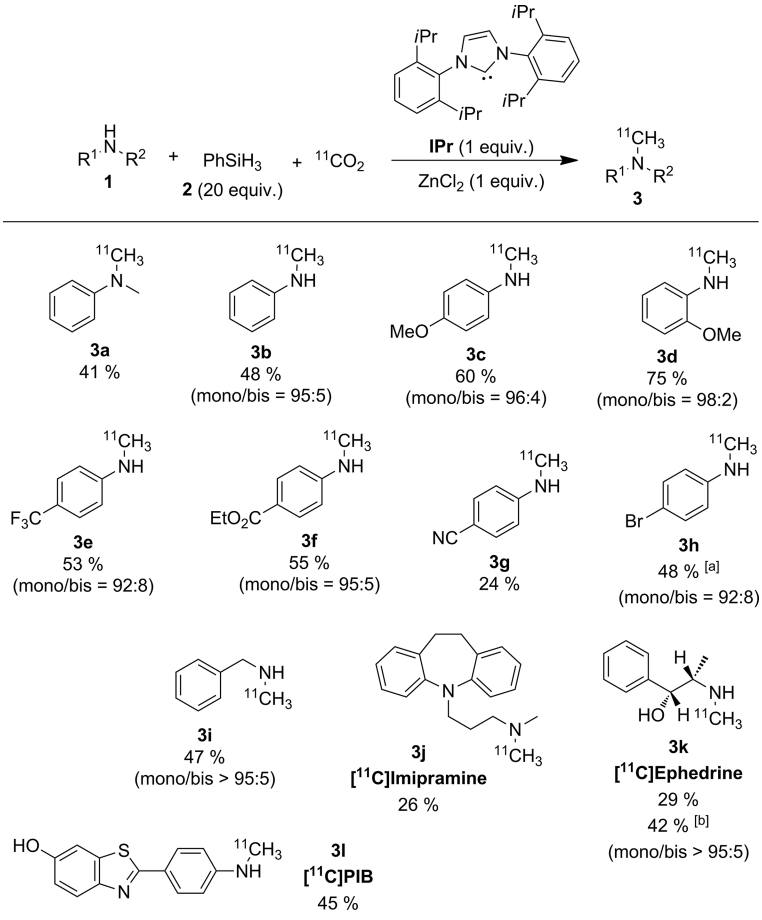
[a] Reagents and conditions: Solvent (400 μL), $ZnCl_2$ (1.3 mg), IPr (3.5 mg), $PhSiH_3$ (23 μL). [b] Radiochemical yields (RCY) were estimated from trapped $^{11}CO_2$ within the reactor and are decay-corrected from the end of $^{11}CO_2$ trapping inside the reactor. [c] $ZnCl_2$ (3.5 mg), IPr (7 mg). [d] Without $ZnCl_2$ and IPr. [e] Without IPr. [f] Without $ZnCl_2$. [g] With pre-formed complex [IPr·$ZnCl_2$] (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 a similar catalytic system in the same solvent and at the same temperature as used in the optimal described conditions, only 3% of the expected methylation was observed in 20 min (entry 1). Such a disappointing result can be rationalized by the short reaction time, imposed by the ^{11}C half-life, compared with the 20 hours required in the original publication. To increase the reaction kinetics, a solvent with similar properties and polarity but with a higher boiling point was selected to increase the reaction temperature. Thus, in diglyme at 150 °C, a satisfactory radiochemical yield of 40% was obtained (entry 2). Because of the small molar amount of $^{11}CO_2$ generated by the cyclotron (e.g., 1 Ci, i.e., 37 GBq, corresponds to 108 pmol), the precursor (**1a**) quantity was decreased to match the standard amount (ca. 1–2 mg) used in classic radiolabeling without any significant change in yield (entry 3). It should be noted that, for practical reasons (weighing), the same amount of catalyst was preserved, bringing the catalyst amount to a stoichiometric level (relative to **1a**). With a shortened reaction time of 10 min, the radiochemical yield was halved (entry 4), even with twice the amount of catalyst (entry 5). The presence of both $ZnCl_2$ and IPr proved to be essential (entries 6–8). However, preliminary complex formation [$ZnCl_2$·IPr][14] appears to be deleterious for radiolabeling, in contrast with the work of Cantat and co-workers (entry 9).[13b] To increase $^{11}CO_2$ concentration in solution to potentially accelerate the reaction, TMEDA (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine) as CO_2 trap was added, as described by Pike and co-workers.[7] However, no reaction was then observed: TMEDA certainly competes with **1a** to catch CO_2 (entry 10). Finally, microwave irradiation also appears to be detrimental for the reaction; the increased molecular agitation contributes to breaking the amine–CO_2 interaction, which seems to be essential to initiate the methylation (entry 11). Furthermore, Dyson and co-workers described a similar methylation under metal-free conditions in the presence of another carbene, namely NHC [1,3-bis(2,4,6-trimethylphenyl)-1,3-dihydro-2*H*-imidazol-2-ylidene].[13e] Unfortunately, under our conditions, the expected radiolabeling was not observed (entry 12).

Moreover, the trapping efficiency of cyclotron-produced $^{11}CO_2$ was measured under the optimal conditions (Table 1, entry 3) at a trapping flow rate of 10 mL/min. Untrapped $^{11}CO_2$ was confined within an Ascarite® cartridge attached to the reactor outlet. Up to 80% of the delivered $^{11}CO_2$ remained inside the reactor at the end of entrapment (trapping time: 60 s). More precisely, the trapping efficiency depends on the basicity of the amine: 80% with **1d**, 70% with **1a**, and 65% with **1e**.

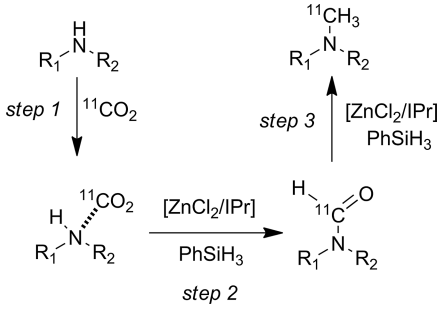
With the optimal reaction conditions in hand (Table 1, entry 3), the scope of this new radiolabeling strategy was investigated using various amines (Scheme 1).

These [11C]methylation reactions generally gave satisfactory radiochemical yields with aromatic or aliphatic amines. Primary amines were selectively monomethylated and, generally, only a small amount of dimethylation was observed. With anilines, the presence of electron-withdrawing or -donating substituents seemed to have no relevant influence (**3b**–**3h**). In accordance with the supposed mechanism (Scheme 2), electron-withdrawing groups should favor the first step of CO_2 trapping by the amine, whereas electron-donating substituents should more favor the second and third reduction steps. The reaction is also compatible with the ester function (**3f**). In the case of the cyano group, a more moderate yield was observed, maybe due to the partial chelation of $ZnCl_2$ by the nitrogen atom partially quenching the reaction. Starting from *p*-bromoaniline (**1h**), a satisfactory yield of the expected product was obtained, despite the formation of **3b**, which arises from the partial reduction of the C–Br bond. This radiolabeling also appears to be chemoselective because in the case of amino alcohol **1k**, only *N*-methylation was observed (see **3k**). However, to achieve similar radiochemical yields compared with other amines, an excess of the amino alcohol (vs. $ZnCl_2$) was required. This could be explained by chelate formation between the amino alcohol and zinc, which could deactivate the nitrogen atom and thus would disfavor the



Scheme 1. Direct [^{11}C]methylation of amines with $^{11}CO_2$. RCY values are mean (n = 3). They were estimated on the basis of trapped $^{11}CO_2$ within the reactor and are decay-corrected from the end of $^{11}CO_2$ trapping inside the reactor. [a] Ratio **3h**/**3b** = 60:40. [b] With 2 equiv. of **1k** vs. $ZnCl_2$.

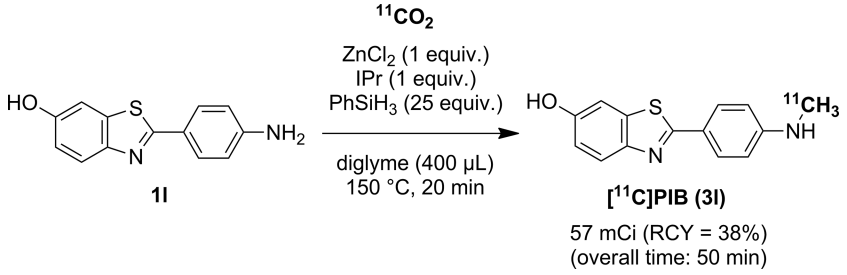
trapping of CO_2. Finally, this methodology can be applied to the synthesis of radiolabeled drugs, such as ephedrine (sympathomimetic amine, **3k**), imipramine (tricyclic antidepressant, **3j**), and the β-amyloid radiotracer [^{11}C]PIB (Alzheimer disease diagnostic, **3l**).



Scheme 2. Hypothesized mechanism for the [^{11}C]methylation with $^{11}CO_2$.

In terms of mechanism, Cantat and co-workers proposed the over-reduction of an intermediate formamide (Scheme 2, steps 2 and 3).[13b] Furthermore, radiomethylation by reduction of a formamide has been previously reported.[15] Under our conditions, the CO_2–amine interaction appeared to be determining. Indeed, an increase of molecular agitation by microwave irradiation[16] disfavored the reaction by breaking this interaction. In addition, starting from imipramine hydrochloride **3j**, no labeling was observed due to the impossibility of the CO_2–amine interaction. During the radiomethylation of **1a**, the corresponding labeled formamide could be identified by HPLC. These observations led us to propose the pathway shown in Scheme 2: formation of the CO_2–amine complex, then first reduction to formamide, and thereafter second reduction to the expected methylamine.

This direct ^{11}C-labeling strategy was applied to the effective production of the β-amyloid radiotracer [^{11}C]PIB[17] (**3l**) with HPLC purification and a final formulation step (Scheme 3). [^{11}C]PIB was produced in sufficient radioactive quantity (57 mCi, RCY = 38%) with a high radiochemical and chemical purity. Such a result is very encouraging and demonstrates the possibility of extrapolating this new strategy to a "productive scale" of radiotracers. Specific radioactivity, however, remained weak (SA = 15 GBq/μmol), certainly due to excess of environmental CO_2 which dilutes $^{11}CO_2$. Nevertheless, in a classical production of [^{11}C]PIB (the [^{11}C]methyl triflate method) with the same synthesizer



Scheme 3. Production of β-amyloid radiotracer [11C]PIB. The RCY value was estimated on the basis of trapped $^{11}CO_2$ within the reactor and is decay-corrected from the end of $^{11}CO_2$ trapping inside reactor.

and a similar production of $^{11}CO_2$ by the same cyclotron, [11C]PIB was obtained with a similar radiochemical yield of 45% and a specific activity of around 50 GBq/µmol.

Conclusions

We have demonstrated the proof-of-concept of direct and selective amine [11C]methylation by the direct use of cyclotron-produced $^{11}CO_2$. 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 [11C]PIB (3l): Cyclotron-produced $^{11}CO_2$, 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 a reactor containing $ZnCl_2$ (1.3 mg), IPr (3.5 mg), and **1l** (1.7 mg) in diglyme (400 µL) and $PhSiH_3$ (23 µL) cooled to 0 °C ($^{11}CO_2$ traps: 711 mCi). The Teflon® lines were removed when the radioactivity content reached a maximum and the reacting mixture was heated at 150 °C for 20 min. After cooling, the HPLC mobile phase (2.2 mL) was added to the mixture and the resulting solution was purified by HPLC on a Waters Symmetry-Prep C18 column (7 µm, 7.8 × 300 mm) at a flow rate of 4 mL/min (H_2O/MeCN, 60:40, v/v). The [11C]PIB fraction (t_R = 11 min) was collected, diluted in water (40 mL), and formulated by solid-phase extraction (SPE). After rinsing with water (10 mL), the purified product was released from the Sep-Pak (Waters Plus tC18) with ethanol (1 mL) and water (2 mL) in a sterile vial (57 mCi).

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