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Published in:
Chemical communications (Cambridge, England)

DOI:
10.1039/d0cc05392a

Publication date:
2021

ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2021, 57, 203

Received 7th August 2020,
Accepted 26th November 2020

DOI: 10.1039/d0cc05392a

rsc.li/chemcomm

Cross-coupling of [^{11}C]methyllithium for ^{11}C-labelled PET tracer synthesis†

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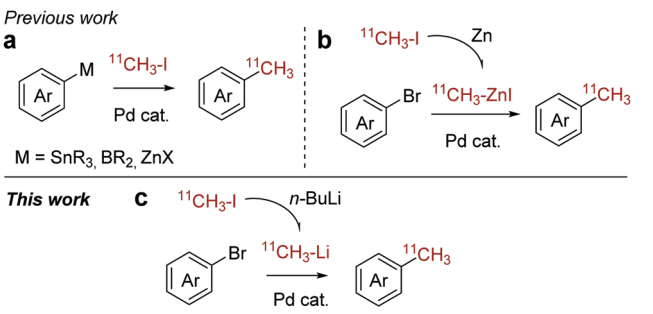
The cross-coupling of aryl bromides with [^{11}C]CH_3Li for the labelling of a variety of tracers for positron emission tomography (PET) is presented. The radiolabelled products were obtained in excellent yields, at rt and after short reaction times (3–5 min) compatible with the half-life of ^{11}C (20.4 min). The automation of the protocol on a synthesis module is investigated, representing an important step towards a fast method for the synthesis of ^{11}C-labelled compounds for PET imaging.

Positron emission tomography (PET) relies on the synthesis of radioactive tracers to image (patho)physiological processes. ^{11}C is a suitable radionuclide for labelling of such tracers, but its short half-life (20.4 min) represents a major hurdle in the development of new labelling strategies. So far, the vast majority of radiolabelling with ^{11}C is performed *via* nucleophilic substitution reactions using [^{11}C]CH_3I[1] or [^{11}C]CH_3OTf,[2] as well as carbonylation reactions using [^{11}C]CO and [^{11}C]CO_2.[3] While these procedures allow for ^{11}C-heteroatom and sp^3–sp^3 bond formation, cross-coupling reactions offer unique opportunities for creating ^{11}C-tolyl functionalities, thereby drastically expanding the current labelling possibilities. Cross-coupling reactions to introduce ^{11}C have been explored,[4] as illustrated by Sonogashira,[5] Heck,[6] or Suzuki[7] coupling reactions (Scheme 1a). A common drawback of these transformations is their inherently low reactivity, which limits their use to a few privileged substrates. As a result, scarcely any of the above mentioned ^{11}C-cross-coupling reactions are employed in routine clinical production. A more versatile protocol involves Stille coupling, which was first used in 1995 by Andersson *et al.*[8] and was then applied to synthesize a wide variety of clinically relevant PET tracers.[9] Nevertheless, Stille coupling reactions involve highly toxic tin reagents and are generally carried out at elevated temperatures, which often result in moderate to low radiochemical yields. Recently, a promising cross-coupling of [^{11}C]CH_3ZnI has been reported (Scheme 1b), although further studies toward relevant tracers are necessary to assess its full potential.[10] Therefore, there is a clear need for alternative, fast and versatile methods for ^{11}C–C bond formation to provide access to new classes of ^{11}C-labelled compounds for clinical use.

Taking advantage of the reactivity of organolithium reagents,[11] [^{11}C]CH_3Li was reported to introduce ^{11}C employing nucleophilic substitution reactions and synthesized from [^{11}C]MeI (production detailed in the ESI†). The high reactivity of [^{11}C]CH_3Li allowed for short reaction times, enabling direct, as well as indirect, labelling.[12] Recent reports extensively studied the cross-coupling with non-radioactive organolithium reagents (including MeLi) to provide greener, faster (down to 5 s reaction time under ambient conditions), and highly selective catalytic methods. Therefore, the general scope, solvent effects, functional group compatibility, and the potential of this protocol for the synthesis of a variety of compounds has been established,[13] as well as its applicability in the radiosynthesis of [^{11}C]Celecoxib.[14] This novel reactivity in cross-coupling reactions broadens the potential applications of [^{11}C]CH_3Li for



Scheme 1 Approaches to ^{11}C labelling *via* cross-coupling.

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† Electronic supplementary information (ESI) available: General, experimental procedures and characterisation of compounds. See DOI: 10.1039/d0cc05392a

direct labelling, and was envisioned as a useful methodology for radiolabelling of PET tracers (Scheme 1c). Here, the versatility of this synthetic approach is assessed through cross-coupling-based radiolabelling with $[^{11}C]CH_3Li$ of three relevant classes of PET tracers: PiB-like compounds, estradiol derivatives and vesamicol derivatives. These tracers may have future potential in amyloid detection, breast cancer imaging and for early diagnosis of neurodegenerative diseases, respectively.

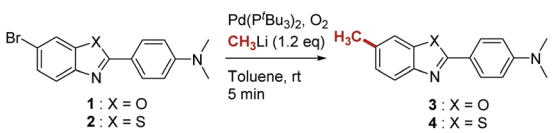
[^{11}C]Pittsburgh Compound B (PiB) is used routinely in the clinic for the imaging of β-amyloid sheets in the brain, enabling early diagnosis of Alzheimer's disease.[15] Besides PiB, several other compounds containing benzothiazole or benzoxazole cores exhibit good affinity for amyloid plaques.[16] Moreover, benzothiazole and benzoxazole heterocycles are present in a range of drugs with various applications such as anti-tumour or anticonvulsant agents,[17] thereby representing an interesting target for labelling.

First, the cross-coupling reaction of the aryl bromides of interests was optimized with CH_3Li to afford the reference compounds. When subjecting bromo-benzoxazole **1** to the cross-coupling reaction, employing catalytic conditions previously described (5% Pd(P^tBu_3)$_2$ oxidized for 30 min),[13] no conversion was observed (Table 1, entry 1), probably due to incomplete conversion of the Pd(P^tBu_3)$_2$ into the oxygen-activated catalyst. Aiming for full oxidation, a solution of Pd(P^tBu_3)$_2$ in toluene was stirred under O_2 atmosphere for 24 h before it was added to the reaction mixture. With this highly active catalyst, full conversion of **1** was achieved in 5 min at ambient temperature, and the reference compound **3** was obtained in 87% isolated yield (Table 1, entry 2). Notably, the oxidized active catalyst solution did not lose its catalytic activity upon storage for least several weeks under dry conditions. In the case of the bromo-benzothiazole **2**, no product formation was observed using 5% of activated catalyst (Table 1, entry 3). This lack of reactivity was attributed to the coordination of palladium with the thiazole moiety, in accordance with previous reports that describe thiazoles as excellent coordinating ligands for several metals.[18] Therefore, an additional Lewis acid was envisioned to strongly coordinate to **2**, allowing the catalytic reaction to occur. Indeed, upon addition of $BF_3{\cdot}Et_2O$, the conversion toward the coupled product **4** increased drastically (Table 1, entries 4 and 5). Alternatively, a higher catalyst loading could be used to compensate for the reduced reactivity in the presence of coordinating moieties (Table 1, entry 6).

Another clinically relevant class of tracers selected to test the fast introduction of ^{11}C *via* CH_3Li cross-coupling are estradiol analogues, which are known to bind to estrogen receptors. They proved to be useful in molecular imaging, especially since the introduction of 16α-[^{18}F]fluoro-17β-estradiol for the detection of breast cancer.[19] The cross-coupling between brominated estradiol **5a** and CH_3Li was unsuccessful in the presence of free alcohol and phenol moieties (Table 2, entry 1), but proceeded fast and in excellent yield with protected alcohols (Table 2, entry 2), leading to the 2-methyl estradiol **6a** after deprotection. *Tert*-Butyldimethylsilyl (TBDMS) was chosen as protecting group, as it offers the perfect balance between stability under the reaction conditions and easy deprotection, which was achieved in 2 min under acidic conditions. The efficiency of the cross-coupling with **5b** also demonstrates that crowded aryl bromides are suitable substrates, as the coupling reaction takes place in 5 min, *ortho* to the bulky TBDMS protecting group.

Encouraged by these results, we focused on PET tracers for an entirely different clinical target and investigated the reactivity of vesamicol compounds towards the organolithium cross-coupling. Vesamicol derivatives have been identified as ligands for vesicular acetylcholine transporters (VAChT)[20] and became leading compounds in the quest for early diagnosis of neurodegenerative diseases.[21] Therefore, numerous derivatives based on this lead

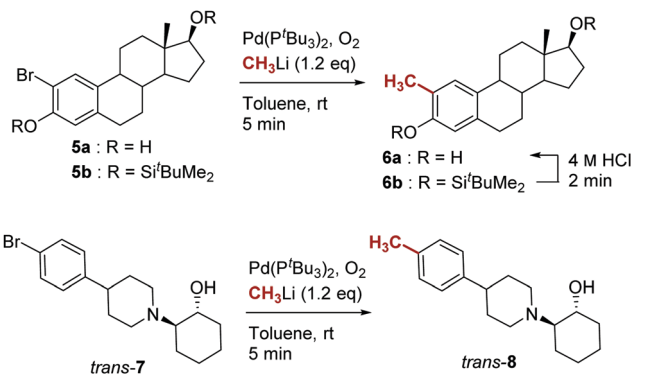
Table 1 Optimization of the cross-coupling reaction for potential amyloid imaging agents



Entry	Substrate	Catalyst	Additive	Conv. (%)[a]
1	1	5% ox. 30 min	None	0
2	1	5% ox. 24 h	None	>99 (87)
3	2	5% ox. 24 h	None	0
4	2	5% ox. 24 h	1 eq. $BF_3{\cdot}Et_2O$	50
5	2	5% ox. 24 h	2 eq. $BF_3{\cdot}Et_2O$	85 (79)
6	2	10% ox. 24 h	1 eq. $BF_3{\cdot}Et_2O$	90

Reaction conditions: Aryl bromide (0.5 mmol) in toluene (2 mL). Additive used if necessary. Pre-oxidized catalyst added as a 10 mg mL^{-1} solution in toluene. Organolithium reagent was added over 5 min, followed by quenching with MeOH. (a) Conversion obtained from GC-MS analysis, yield of isolated product is given in parentheses.

Table 2 Optimization of the cross-coupling to access products with hydroxyl functionalities



Entry	Substrate	Conv. (%)[a]
1	5a	0
2[b]	5b	>99 (90)
3[b]	*trans*-7	>99 (85)

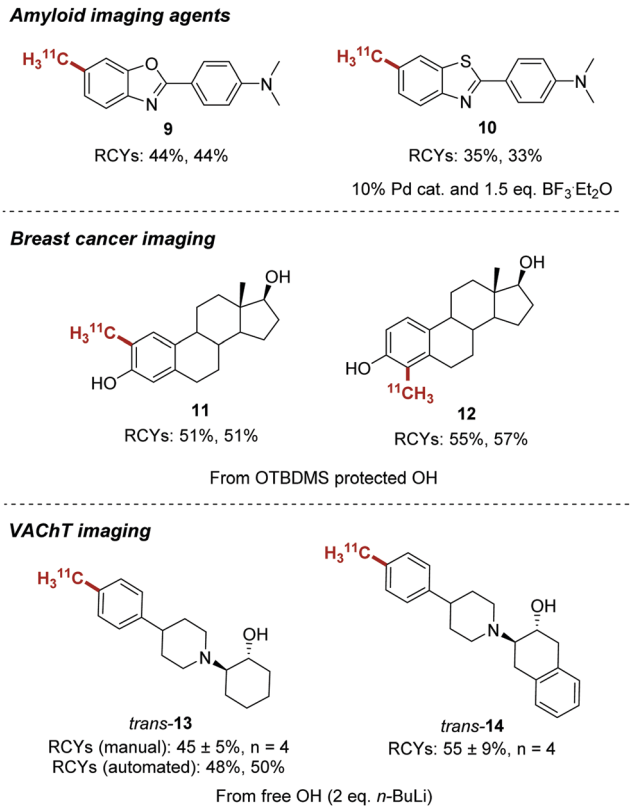
Reaction conditions: aryl bromide (0.5 mmol) in toluene (2 mL). Catalyst pre-oxidized 24 h (5 mol%) added as a 10 mg mL^{-1} solution in toluene. Organolithium reagent added over 5 min, followed by quenching with MeOH. (a) Conversion obtained from GC-MS analysis, yield of isolated product is given in parentheses. (b) Hydroxyl functionalities deprotonated with *n*-BuLi prior to catalyst addition.

structure were synthesized and evaluated *in vitro*,[22] leading to the development of [^{18}F]fluorethoxybenzovesamicol, a tracer applied in the clinic for specific mapping of VAChT.[23] Moreover, vesamicol can also bind to Sigma receptors,[24] whose targeting is of great significance as they play a role in psychiatric disorders.[25] Therefore, a reliable and efficient labelling method for such substrate is highly desirable. When performing the cross-coupling reaction of precursor **7** with CH_3Li, it was observed that protection of the alcohol is not necessary (Table 2, entry 3), in contrast to what was observed for estradiol substrates. The cross-coupling could be performed directly on the lithium alkoxide, formed *in situ* by the addition of 1 eq. of *n*-BuLi, affording *p*-methyl-*trans*-vesamicol **8** in 85% isolated yield (5 min reaction time at rt). In this case, the lithium alkoxide formed is remote from the reactive site, thereby not having the detrimental effect previously observed on the cross-coupling reaction.

With the optimal conditions in hands for each of the substrates, their radiolabelling was pursued. Radiolabelled compounds were prepared by a cross-coupling reaction with [^{11}C]CH_3Li, which was obtained *via* lithium-halogen exchange by bubbling the standard ^{11}C-reagent [^{11}C]CH_3I into a solution of *n*-BuLi in dry toluene (see ESI,† for experimental details). The radiolabelled products obtained are presented in Scheme 2.

Potential amyloid imaging agents **9** and **10** were isolated in good radiochemical yields, respectively 44% RCY and 34%, excellent radiochemical purity of >99%, and with molar activity in the usual range for ^{11}C-PET-tracers (2.2 ± 1.2 GBq μmol^{-1}). Compound **10** was previously reported as a labelled compound [^{11}C]6-Me-BTA-2, where the radionuclide was incorporated at the methylamine position. The suitability of this molecule as PET tracer was evaluated *in vitro*, showing good binding affinity (K_i = 64 nM) with aggregated Aβ fibrils.[26] By introducing a ^{11}C-methyl group directly on the heterocyclic core, we provide a complementary strategy to introduce ^{11}C, allowing for potential modifications on the amine part. Using the above described conditions, [^{11}C]methyl-estradiols **11** and **12** were synthesized and isolated in excellent radiochemical yields (≥51%) and radiochemical purities (>99%). Notably, the overall reaction time could be kept to 5 min, including the deprotection step. To the best of our knowledge, this is the first reported radiolabelling method for these molecules. Steroid **11** was previously identified as an estradiol agonist, with improved metabolic stability, capable of significantly downregulating estrogen receptor production and upregulation the production of progesterone receptors.[27] Binding affinity data gathered on the non-radiolabelled analogue of **12** indicates a preference for estrogen receptor β (IC_{50} = 25 nM) over subtype α (IC_{50} = 125 nM).[28] This 5-fold difference suggests a possibility for selective imaging of estrogen receptor β using compound **12**. The cross-coupling methodology was also successfully applied to vesamicol, affording the isolated *p*-[^{11}C]methyl-*trans*-vesamicol analogue **13** in high RCY. The labelled product **13** was previously prepared by Stille coupling and its (+)-enantiomer showed specific uptake for Sigma-1 receptors, both *in vitro* and in rodent brains.[29] It should be emphasized that, compared to the reported Stille coupling, our protocol exhibits much higher radiochemical yields (7.7 to 19% *via* Stille coupling *vs.* 45 ± 5% reported here) and proceeds at lower temperature (80 °C *vs.* rt). By providing a more efficient route towards a selective Sigma-1 tracer, we demonstrate the potential of the labelling by cross-coupling of [^{11}C]CH_3Li to provide clinically relevant ^{11}C-PET tracers. As shown for FEOBV, benzovesamicol type of structures exhibits better selectivity towards VAChT than vesamicol. Therefore, the labelling of a benzovesamicol scaffold to obtain the *p*-[^{11}C]methyl-*trans*-benzovesamicol **14** was also performed. Again, the radiolabelled compound was isolated in high yield (55% RCY) with a radiochemical purity >99%. By offering a ^{11}C alternative to the established ^{18}F-labelling methods available for these structures, we facilitate the development of ^{11}C-tracers, which are notably advantageous by reducing the radiation dose, especially when performing repeated studies.

In order to transform the above described radiochemical cross-coupling reaction into an ''easy to use'' labelling method, automation of the manual protocol was explored using the cassette-based synthesis module Eckert & Ziegler (see ESI,† for details). The possibility of performing this reaction in an automated module, which is commercially available and complies with good manufacturing practices (GMP), is an important step towards its use in preclinical and clinical PET-studies. Initially, direct translation of the manual protocol to the automated module did not lead to product formation, as



Scheme 2 Radiolabelling with [^{11}C]CH_3Li of relevant PET tracers.

most of the lithium reagent was found to be reactive in contact with the plastic tubing and connections of the cassette. However, increasing the amount of *n*-BuLi from 1.0 to 1.6 eq. circumvented the loss of the radioactive [^{11}C]CH_3Li in the system, thereby recovering similar conversion (up to 50% towards **13**) to the one obtained by manual labelling. Although the purification was not performed in the automated module, the crucial steps of [^{11}C]CH_3Li formation and subsequent cross-coupling reaction could be successfully automated.

In conclusion, a new labelling methodology using [^{11}C]CH_3Li was developed and successfully applied to the synthesis of various clinically relevant targets, including PiB-like compounds, estradiol analogues and vesamicol derivatives. This procedure fulfils the essential requirements of fast reaction times, mild conditions, high yield and high molar activity for the introduction of ^{11}C. The labelling by cross-coupling of [^{11}C]CH_3Li provided the target molecules in radiochemical yields ranging from 33% up to 66% and high radiochemical purity, within 30 to 40 min from the end of bombardment (EOB) until the isolated compound was collected. Automation of the synthesis was also investigated, facilitating future applications. The novel labelling strategy reported here represents a valuable and highly practical tool for ^{11}C-labelling, *via* cross-coupling reaction, of biomedical PET imaging agents.

B. L. F. acknowledges financial support from The Netherlands Organization for Scientific Research, the Royal Netherlands Academy of Arts and Sciences (KNAW), and the Ministry of Education, Culture and Science (Gravitation program 024.60 1035). Bram Maas is kindly acknowledged for operating the cyclotron and providing the [^{11}C]CH_4.

Conflicts of interest

There are no conflicts to declare.

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