One-Pot Synthesis of [11C]-Labelled Primary Benzamides via Intermediate [11C]Aroyl Dimethylaminopyridinium Salts

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Abstract: Electrophilic [11C]-labelled aroyl dimethylaminopyridinium salts, obtained by carbonylative cross-coupling of aryl halides with [11C]carbon monoxide, were prepared for the first time and shown to be valuable intermediates in the synthesis of primary [11C]benzamides. The methodology furnished a set of benzamide model compounds, including the two poly (ADP-ribose) polymerase (PARP) inhibitors niraparib and veliparib, in moderate to excellent radiochemical yields. In addition to providing a convenient and practical route to primary [11C]benzamides, the current method paves the way for future application of [11C]aroyl dimethylaminopyridinium halide salts in positron emission tomography (PET) tracer synthesis.

Introduction

Positron emission tomography (PET) is a non-invasive molecular imaging technique providing spatiotemporal information on molecular processes in living human subjects and experimental animals. A pre-requisite for PET imaging is the efficient radiolabeling of a tracer molecule with a short-lived radionuclide that decays via positron emission.[1–2] Carbon-11 ([11C], $t_{1/2} = 20.3$ min) is an attractive positron-emitting radionuclide for radiopharmaceutical chemistry. The high positron emission decay mode (β^+; 99.8%) and low energy protons (0.96 keV)

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allow for high-resolution *in vivo* imaging. Another advantage is that [11C] often can be introduced in biologically relevant molecules without changing its physicochemical or pharmacological properties, because its stable isotope ([12C]) is the major constituent in organic molecules.[3]

In view of our long-standing objective to facilitate the labeling of druglike molecules for PET imaging, we recently turned our attention to the [11C]-labeling of primary benzamides (Ar—CONH$_2$). The primary benzamide motif is present in a wide range of biologically relevant compounds, including cancer and CNS drugs (Figure 1), but the [11C]-labeling of this motif has received considerably less attention in the literature than that of substituted benzamides, for which there is a number of reliable and efficient methods reported to date. Two transition metal mediated protocols, starting from [11C]cyanide or [11C] carbon monoxide ([11C]CO), have primarily been utilized for labeling of primary benzamides thus far.[4–10] Although these methods provided the target benzamides in high radiochemical yields (RCYs), they suffer from a few drawbacks, including the inconvenient use of toxic ammonia gas as well as the use of high-pressure micro-autoclave equipment.

In a recent study, Arndtsen and co-workers described the synthesis of aromatic amides and esters via carbonylative formation of aroyl dimethylaminopyridinium (aroyl-DMAP) salts, a potent electrophile which is commonly formed *in situ* to facilitate acylation reactions.[11–12] Inspired by this work, we set out to develop an alternative, convenient, method for the preparation of [11C]-labelled primary benzamides. We anticipated that such a method could draw on the recent progress in the development of low-pressure [11C]-carbonylation protocols.[13–17] From a general point of view, any useful new methodology for [11C]-labeling should be late-stage, ideally single pot, and the synthesis procedure should not exceed 2–3 physical half-lives of the employed radionuclide (40–60 min for [11C]).[3]

Results and Discussion

Benzamide (**2**) was selected as a model compound for probing the palladium-mediated [11C]-aminocarbonylation of iodobenzene, with DMAP as an organic additive, and ammonia surrogates as a safer substitute for toxic ammonia gas. In this study, three different commercially available ammonia surrogates were examined, namely, hexamethyldisilazane, ammonium carbamate and formamide. Of these, hexamethyldisilazane and ammonium carbamate turned out to be inefficient (data not shown), on the other hand, the first reaction using formamide furnished [11C]**2** in 48% radiochemical

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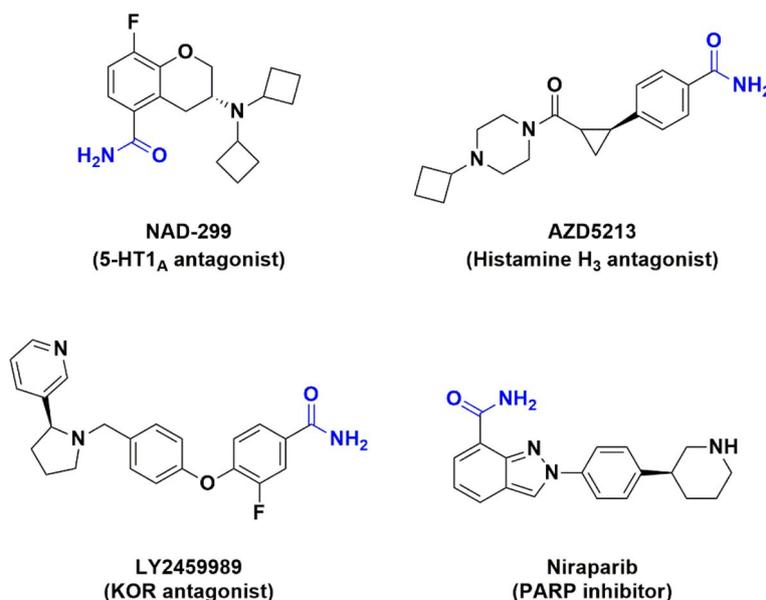European Chemical
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Figure 1. Structures of drug molecules containing the benzamide structural motif.

conversion (RCC) in only 10 min overall synthesis time (Table 1, entry 1), including a 5 min hydrolysis of the intermediate formimide ([^{11}C]**1**), which proceeded in a quantitative RCC. The high trapping efficiency (TE) of [^{11}C]CO observed for this reaction is due the excellent ability of the Pd-xantphos complex to capture CO.[16] In an attempt to improve this RCC, we next explored how different reagent concentrations, solvents and temperature affects the yield of reaction, which is believed to proceeded via an [^{11}C]aroyl-DMAP intermediate (Table 1).[11–12] In support of this hypothesis, we observed a positive correlation between the RCC of [^{11}C]**2** and the concentration of DMAP. The lowest RCC (9%) was obtained without DMAP (Table 1, entry 4) and the highest RCC (91%) with 442 μmol of DMAP (Table 1, entry 3). In this context it should be noted that DMAP was used

at substantially higher concentrations than in previous reports with unlabeled CO,[11] to enable the reaction to proceed at high yields despite the limited reaction time (5 min) which is desirable to avoid extensive decay of short-lived ^{11}C. Following additional investigations into this relationship, it was found that the DMAP concentration could be reduced to half with only a marginal detrimental effect on the RCC (Table 1, entry 2) and this concentration was therefore adopted in the ensuing study. Gratifyingly, the RCC of [^{11}C]**2** was reproducible under entry 2 conditions ($85 \pm 2\%$, n=4) and we next turned our attention to studying the effect of other reaction parameters. First, we observed that RCC of [^{11}C]**2** was dependent on reaction temperature, with the highest RCC observed at 150°C (Table 1, entry 6) and the lowest at 60°C (Table 1, entry 5). However, only

Table 1. Effect of solvent, temperature and DMAP concentration on the formation of [^{11}C]**2**.

Entry	Solvent	Temp [°C]	DMAP (eq.)	TE [%][a]	RCP [%][b]	RCC [%][c]
1	THF	100	19	100	48	48
2	THF	100	52	95	90	85 ± 2[d]
3	THF	100	104	100	91	91
4	THF	100	–	97	9	9
5	THF	60	52	100	57	57
6	THF	150	52	97	93	90
7[d]	THF	100	52	100	70	70
8	CH$_3$CN	100	52	98	77	75
9	Formamide	100	52	92	91	83
10	Formamide	150	52	95	94	89

Reaction conditions: iodobenzene (12.6 μmol), Pd$_2$(μ-cinnamyl)Cl$_2$ (2.2 mg), Xantphos (5.0 mg), DMAP (25 mg), formamide (100 μL) solvent (1.3 mL), See supporting information for further information. [a] Trapping efficiency (TE); the fraction of radioactivity left the crude product after purging with nitrogen. [b] The radiochemical purity (RCP) is determined by radioanalytical HPLC. [c] The radiochemical conversion (RCC) is based on the total radioactivity delivered to the reaction vessel. [d] Average of four experiments. [d] Formamide 60 μL.

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a marginal improvement in the RCC of [11C]**2**, from 85% to 90%, was observed by increasing the temperature from 100°C to 150°C which does not motivate heating at this higher temperature. Next, the formamide concentration was investigated. It was found that a reduction in the amount of formamide from 100 μL to 60 μL resulted in a 27% reduction in the RCY of [11C]**2** (Table 1, entry 7), whereas using formamide as the sole solvent for the reaction resulted in an 83% RCC of [11C]**2** (Table 1, entry 9). Increasing the temperature to 150°C, while keeping formamide as reaction solvent, further improved the RCC of [11C]**2** to 89% (Table 1, entry 10), which is similar to the observation obtained with THF at the same temperature. Finally, it was also noted that the reaction could be performed in acetonitrile, albeit at a slightly lower RCC (Table 1, entry 8). In summary, we selected THF as solvent (with entry 2 conditions) for our further investigations on the versatility of this new

reaction on additional substrates. THF is widely used in transition metal mediated chemistry and its lower boiling point enables evaporation of the reaction solvent prior to isolation of the product using preparative high-performance liquid chromatography (HPLC).

With the improved conditions in hand, we next turned our attention to testing a set of aryl halide substrates in this new reaction (Figure 2). Since aryl bromides are more bench stable than their corresponding iodides, and thus typically more readily available in medicinal chemistry settings, it was gratifying to see that [11C]**2** was produced in a 64% RCC using phenyl bromide as substrate. After evaluating a set of substituted arenes in the reaction, we made the following key observations; first of all, the reaction proceeded with low yields, if at all, when unprotected anilines were present on the aromatic ring ([11C]**11**–**12**). However, when the amine was N-boc-protected, a
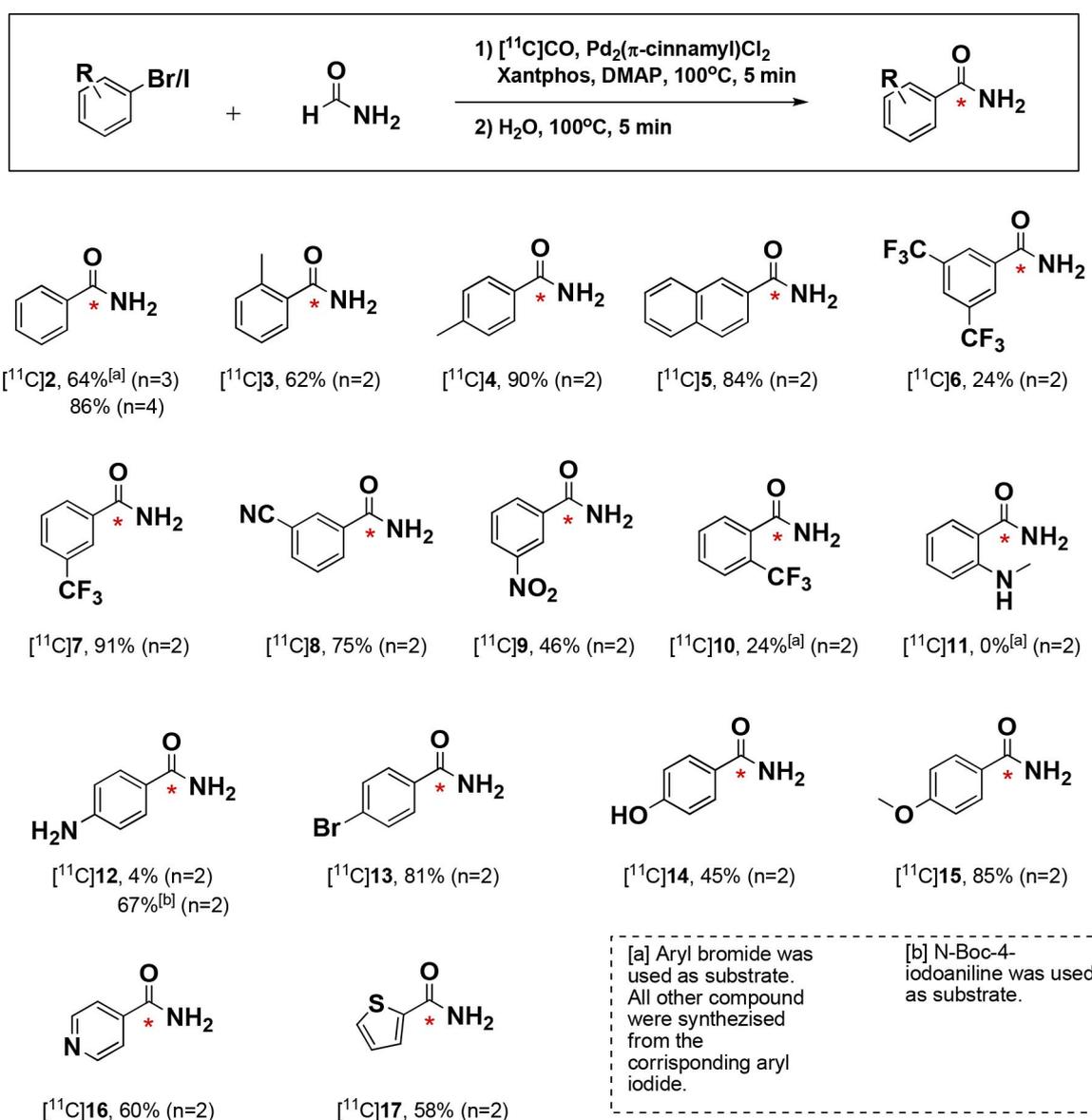

Figure 2. 11C-labelled benzamides formed via intermediate [11C]aroyl-DAMP salts.

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dramatic improvement in RCC was observed ([^{11}C]**12**[b]). Secondly, nitro-, trifluoromethyl- or cyano groups provided good to excellent yields of the corresponding benzamides ([^{11}C]**6**–**10**), thus demonstrating that electron withdrawing groups were well tolerated in the reaction. In this context, it is noteworthy that even the di-trifluoromethyl substituted ^{11}C-labelled carboxamide was furnished in a useful RCC ([^{11}C]**6**). The only exception to this rule was ortho-substituted trifluoromethyl groups that only provided the desired benzamide product in low yield ([^{11}C]**10**). Although it should be noted that a less reactive aryl bromide was used as substrate in this particular reaction, it cannot be ruled out that steric hindrance may also have a detrimental effect on the reaction yield, in particular since a 30% lower yield was observed for the o-tolyl derivative [^{11}C]**3** compared to its p-tolyl analog [^{11}C]**4**. Thirdly, heterocycles and heteroatoms were also well tolerated, as shown by the high RCCs observed for the unprotected phenol and the p-bromine-, methoxy-, thiophene- and pyridine derivatives ([^{11}C]**13**–**17**). Finally, as expected, the reaction proceeded in excellent RCC with neutral or electron rich arenes, as illustrated by the naphthyl-, anisole- and tolyl substituted ^{11}C-labelled benzamides ([^{11}C]**3**–**5**).

To showcase the utility of the new methodology, we set out to radiolabel two benzamide poly (ADP-ribose) polymerase (PARP) inhibitors, namely, niraparib and veliparib, which both have sub-nanomolar affinity for the PARP enzyme.[18] Based on observations from the substituent tolerance studies, we devised a two-step one-pot procedure for their radiolabeling, starting from their N-Boc-protected iodoarene derivatives (Figure 3). It is worth noting that the aqueous hydrolysis step could be eliminated in this setting, possibly due to the use of non-anhydrous TFA for the deprotection. The radiosynthesis of these drug molecules was performed according to good manufacturing practice (GMP) on a recently developed fully automated and commercially available radiochemistry system for low pressure radiocarbonylation.[19] Gratifyingly, both compounds were radiolabeled in sufficient quantity and quality to enable preclinical studies in non-human primates. Thus, following a full cyclotron production (55 μA for 30 min), 541 MBq of [^{11}C] veliparib was obtained at a molar activity (A$_m$) of 9.9 GBq/μmol

and a radiochemical purity exceeding 99%. The isolated radiochemical yield of [^{11}C]niraparib was somewhat higher – 1422 MBq of the final product was obtained, at a A$_m$ of 10.0 GBq/μmol and a radiochemical purity exceeding 99%.

Conclusion

A new method that enabled a range of primary benzamides to be labelled with carbon-11 in good to excellent yields was developed. The successful application of the new methodology to the radiolabeling of two drug molecules in a commercially available synthesis module, and according to GMP, paves the way for future applications in the preparation of new PET tracers for the clinic. Overall, this current work introduces an efficient method to create electrophilic [^{11}C]aroyl-DAMP salts, a new class ^{11}C-labelled carbonylation products for PET radiopharmaceutical synthesis.

Experimental Section

General procedure for ^{11}C-labeling of benzamides via intermediate formation of [^{11}C]aroyl dimethylaminopyridinium salts: Cyclotron produced [^{11}C]CO$_2$ are reduced on-line to [^{11}C]CO over heated Mo powder. The produced [^{11}C]CO is first concentrated on a silica gel trap immersed in liquid nitrogen before it is transferred into the sealed reaction vessel containing the coupling reagents (Pd$_2$[π-cinnamyl]Cl$_2$ (2.2 mg), xantphos (5,0 mg), iodobenzene (1.4 μL), DMAP (27 mg), formamide (100 μL) dissolved in anhydrous THF (1.3 mL). The resulting mixture was allowed to react for 5 min at 100°C followed by another 5 min reaction at 100°C after the addition of water (500 μL). The radioactivity was measured before and after the vial was purged with nitrogen. RCP of the crude reaction mixture was established with radio-HPLC. For more detailed description of the reaction procedure see the supporting information.

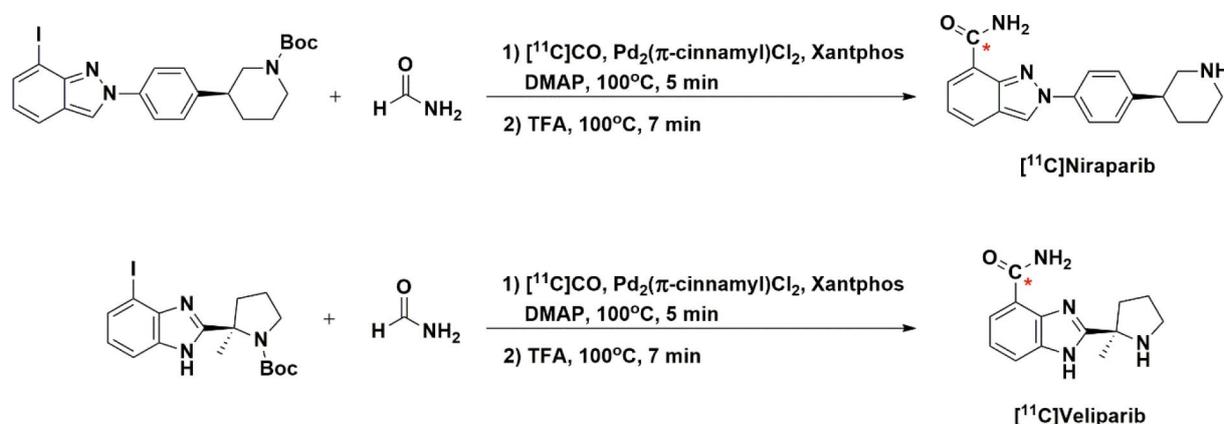**Supporting Information**: General methods, experimental procedure, Radio-HPLC traces for all labelled compounds.

Figure 3. Synthesis of the two radioactive druglike molecules [^{11}C]niraparib and [^{11}C]veliparib.

Conflict of Interest

The authors declare no conflict of interest.

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