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Accelerating Preclinical PET-Screening: Reductive Amination with [^{11}C]Methoxybenzaldehydes

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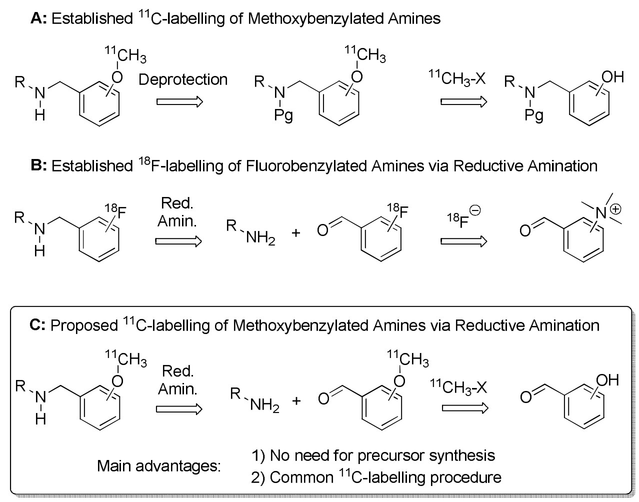
We report, herein, a simple and efficient labelling strategy for multiple PET tracer preparation using a common intermediate , which has the potential to accelerate preclinical PET radiotracer screening. This procedure was applied to and compared with a previously published labelling strategy illustrating the advantages of this newly developed combinatorial approach.

Positron emission tomography (PET) is a powerful non-invasive tool to characterize, e.g., receptors, enzymes and other targets in vivo. For example, it can be used to quantify receptor binding or receptor occupancy of a given drug at a specific target. For drug discovery processes or disease diagnosis, these outcomes are extremely useful to inform drug development or treatment decisions[1].

PET tracer development is usually guided by medicinal chemistry structure activity relationship (SAR) studies, where affinity, selectivity towards a certain target and lipophilicty is optimized. Based on these, a few compounds are selected, subsequently labelled and finally evaluated[1d]. Carbon-11 ($t_{1/2}$ = 20.4 min) is often the chosen nuclide for development research due to the possibility to conduct repeated PET studies in the same subject within hours[2]. Traditionally, a last-stage ^{11}C-methylation strategy is applied[3], whereas multi-step syntheses involving ^{11}C-labelled intermediates are thought to be inferior due the short half-life of carbon-11 and thus lower radiochemical yield (RCY).

We recently developed a carbon-11 labelled radiotracer for the 5-HT$_{2A}$ receptor[4]. During the development phase, we screened 12 ligands synthesised from a classical labelling approach of anisols. This required the synthesis of specific precursors for each tracer resulting in 24 additional precursor synthesis steps (Scheme 1A). Therefore, we became interested in developing a new approach that would provide ready access to this class of tracers and circumvent the need for arduous precursor synthesis. Inspired by the use of ^{18}F-labelled benzaldehydes for ^{18}F-labelling of related compounds (Scheme 1B)[5], we aimed to develop a similar procedure based on an early-stage ^{11}C-labelling of a simple fragment, which could subsequently be coupled to any amine via a reductive amination step (Scheme 1C).



Scheme 1: A: Classical approach to labelling of anisole derivatives requiring protection of amines. B: ^{18}F-labelling via formation of ^{18}F-benzaldehydes follow by reductive amination of a primary amine. C: Proposed fusion of the former protocols.

We believe that such a 2-step radiosynthesis is advantageous compared to late-stage labelling - in particular in the preclinical screening phase, because necessary precursor synthesis steps are minimized since the needed amines already have been prepared during the synthesis of the target compounds. Although this labelling approach has been reported in the literature, it has not been applied to the synthesis of a library of PET-ligands as detailed herein [6].

Computational approaches to predict the in vivo behaviour of radiolabelled compounds based on in vitro characteristics are unreliable [1d, 7], and the development of new PET-tracers is still largely a “trial-and-error game”.

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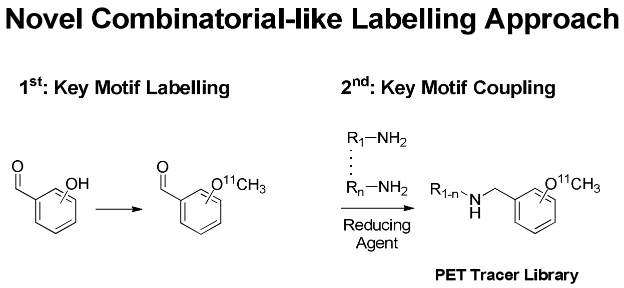


Scheme 2: This combinatorial labelling approach could provide easy access to a PET tracer library for in vivo preclinical evaluation studies by circumventing precursor synthesis for every single PET tracer.

For example, we recently demonstrated that kinetics, non-specific binding and ultimately the binding potential of 9 structurally related phenethylamines differed quite dramatically, even though the in vitro profiles were comparable[4]. Others have also found that small molecular changes to the lead compound have profound effects on the compounds behaviour as a PET-ligand.[8] Thus, rather than just labelling the ligand from a compound series with the best in vitro profile, one should investigate several representatives from the same compound class. Ready access to structurally similar PET-ligands with similar pharmacological in vitro profiles greatly increases the chance of success.

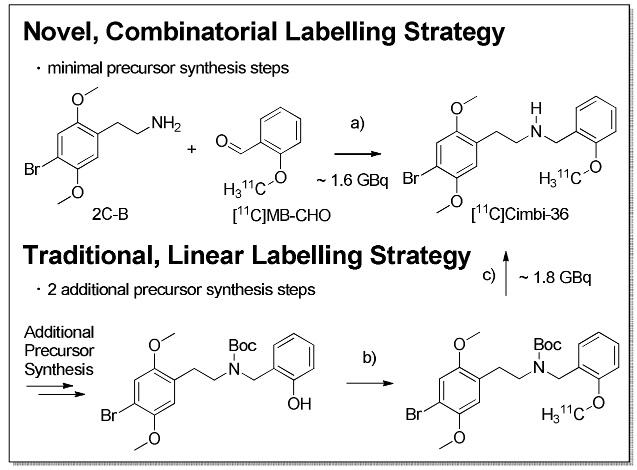
Only a limited number of studies have been published conducting combinatiorial-like multi-step approaches to create PET tracer libraries[9]. For example, Långström and coworkers utilized such a strategy by applying palladium mediated ^{11}C-carbonylations[9a-c, 10]. This is surprising since a fast and efficient PET tracer access would facilitate the preclinical evaluation process and probably increase the success rate of novel tracers reaching the clinic.
Therefore, our main goal was to extend the existing combinatorial-like strategy to a 2-step key motif ^{11}C-labelling approach (Scheme 2). Two prerequisites have to be fulfilled for such a procedure.

1) The labelling of the key motif as well as the subsequent coupling has to be fast and efficient (< 10 min, RCY > 50%).
2) The purification and formulation must be efficient and rapid (< 15 min).

Consequently, the total synthesis time should not exceed 40 min and an isolated yield > 300 MBq has to be achievable.

Reductive aminations are fast, efficient and can be conducted chemoselectively[11]. Furthermore, many drugs contain amines and thus a broad variety of target compounds exist which can be synthesized applying this strategy[12].

To develop this approach, we chose to focus on the labelling of [^{11}C]Cimbi-36, a new PET-tracer for the visualisation of the 5-HT_{2A} receptor in the CNS [4]. The reason for that was twofold: Firstly, procedures for radiolabelling methoxybenzaldehydes (MB-CHO) in high radiochemical yields (> 80%) and short reaction time (< 5min) are well described [13].



Scheme 3: Comparison of the direct labelling approach a) with the established labelling procedure via b) and c). a) $NaBH_3CN$, AcOH, DMSO/MeOH, 130 °C, 5 min. b) [^{11}C]CH_3OTf, MeCN/acetone, NaOH, 40 °C, 30 sec, c) TFA, MeCN, 80 °C, 5 min

Secondly, this would allow us to compare the new approach with a known protocol. In scheme 3, the established procedure for ^{11}C-labelling via alkylation of a Boc-protected precursor along with the proposed reductive amination approach is outlined.

The short half-live of carbon-11 puts severe restrictions on the number and kind of transformations one can conduct after the label has been introduced. Ackermann et al. showed that a 2-step reductive amination strategy of a ^{11}C-labelled aniline derivative could be successfully carried out[6a, 14]. They utilized this strategy to circumvent side-product formation otherwise observed using a traditional last-step labelling approach. Furthermore, successful reductive aminations of [^{18}F]fluorobenzaldehyde ([^{18}F]FB-CHO) with various amines have been performed in 10-15 min using sodium cyanoborohydride ($NaBH_3CN$)[5]. Thus, the proposed reductive amination approach should be feasible for a broad spectrum of amines in the short reaction times required for carbon-11 chemistry (< 10 min).

Therefore, we tried to combine the known labelling procedure of 2-[^{11}C]methoxybenzaldehyde (2-[^{11}C]MB-CHO) with the reductive amination conditions for [^{18}F]FB-CHO and applied them to primary phenethylamines[5, 13], which was successful at first. However, several parameters needed optimization: Firstly, a 15-20 minute reaction time was detrimental to the RCY and secondly, since relatively large amounts of the precursors were used (25 mg of 2C-B and 2 mg of 2-hydroxybenzaldehyde (2-HBA)), formation of significant amounts of non-radioactive side-products complicated the subsequent purification of the tracer. Therefore, we aimed to optimize and improve these in the next phase.
No influence of precursor concentration on the radiochemical yield of the first labelling step could be detected. Thus, we could successfully lower the amount of precursor to 5% of the original reported procedure. Furthermore, the amount of 2C-B and $NaBH_3CN$ could be reduced, greatly simplifying the final purification. Thus, a successful separation after a 2-step, one-pot reductive amination was achievable (Table 1).

Table 1: Tested radiolabeling conditions of the novel reductive amination approach for the synthesis of [^{11}C]Cimbi-36

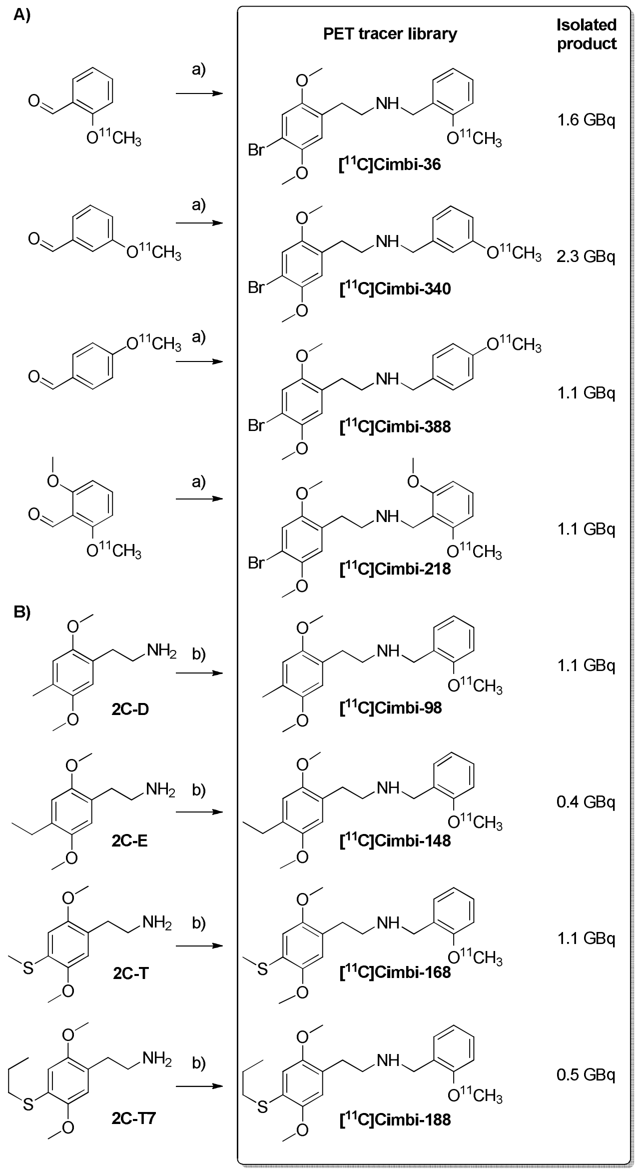
1st Key Motif Labelling (0.3 mL DMSO, [^{11}C]MeOTf)				2nd Key Motif Coupling (0.6 mL MeOH, 5 µL AcOH, 130 °C)			Total Yield*
2-HBA [mg]	2M NaOH [µL]	Time [sec]	Temp. [°C]	2C-B [mg]	$NaBH_3CN$ [mg]	Time [min]	Isolated product [MBq]
2	20	30	25	25	25	5	931
0.3	3	30	25	6	6	5	752
0.1	1	30	25	4	4	5	815
0.1	1	60	25	4	4	5	1684
0.1	1	120	40	4	4	5	226
0.1	1	120	60	4	4	5	230
0.1	1	30	25	4	4	1	20
0.1	1	30	25	4	4	2.5	43

* Isolated yield was determined at end of synthesis, see ESI

It is worthwhile to mention that the use of glacial acetic acid (AcOH) was essential for the 2nd reaction step and that we were not able to reduce the reaction time for the reductive amination to below 5 min. Insufficient imine and subsequently amine conversion was otherwise observed. Moreover, the use of the less toxic reducing agent sodium triacetoxyhydroborate failed. Finally, we optimized the RCY for the 1st labelling reaction, where the use of [^{11}C]CH_3OTf instead of [^{11}C]CH_3I decreased the reaction time from 5 min to 60 s. Eventually, the optimal conditions were found to be: 1st labelling step: [^{11}C]CH_3OTf , 1 µmol 2-HBA, 2 µmol 2N NaOH, 300 µL DMSO, 25 °C and 60 s; 2nd labelling step: 15 µmol 2C-B, 63 µmol $NaBH_3CN$, 87.5 µmol AcOH, 0.6 mL MeOH, 130°C, 300 s. Table 1 summarizes the experiments conducted.

In summary, [^{11}C]Cimbi-36 could be labelled in a simple and efficient 2-step, one-pot synthesis within 40 min (Yield:1.6 GBq, specific radioactivity: 60 - 146 GB/µmol, radiochemical purity: > 99%). Compared with our previous optimized classical 2-step ^{11}C-labelling strategy, the new method usually resulted in ~10-20% lower isolated yield, whereas other parameters were similar. Despite the lowered yield, this novel strategy is more than sufficient to conduct preclinical imaging since usually about 300 MBq is required for an in vivo PET scan in larger animals. In regards to a tracer library labelling approach, the synthesis fulfilled the prerequisites and thus, we tested this method on other structurally related phenethylamine structures. Several ortho-, meta- and para-HBA moieties were successfully labelled and subsequently coupled to 2C-B (Scheme 4A). Moreover, four diverse primary phenethylamines were coupled to 2-[^{11}C]MB-CHO (Scheme 4B). Similar reaction parameters were observed for all conducted experiments and therefore, we believe that this novel combinational 2-component reductive ^{11}C-amination approach is generally applicable to all kinds of key motif reductive amination approaches and leads, indeed, to an easy tracer library access.

In conclusion, a library of eight phenethylamines was labelled without the need for time consuming precursor synthesis. Thus, we have shown that this novel synthetic library approach, via a key radiolabelling intermediate, facilitates preclinical screening by reducing the number of precursor synthesis steps substantially. In a broader perspective, this library approach could be extended to different secondary labelling motifs such as piperazines or piperidines, which are common features in drug molecules.



Scheme 4: Standard reductive ^{11}C-amination conditions ($NaBH_3CN$, appropriate $R-NH_2$, CH_3COOH, DMSO/MeOH, see supporting information for further details.) which resulted in an easy access to 8 PET tracers. A) Coupling of MB-CHO derivatives to 2C-B; B) Coupling of 2-[^{11}C]MB-CHO to different phenethylamine derivatives.

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Notes and references

† Electronic Supplementary Information (ESI) available: Experimental procedures, analytical HPLC conditions, semi-prep HPLC conditions, GMP compliant radiosynthesis for Cimbi-36, spectroscopic data for selected compounds. See DOI: 10.1039/b000000x/

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