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Development of a safe and scalable route towards a tau PET tracer precursor

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ABSTRACT

A scalable 5-step synthesis of the diazacarbazole derivative **1** used as tau PET tracer precursor is reported. Key features of this synthesis include a *Buchwald-Hartwig* amination, a Pd catalyzed C—H activation and a *Suzuki-Miyaura* cross-coupling.

1. Introduction

Beta amyloid (Aβ) plaques and tau aggregates are key histopathological characteristics of Alzheimer's disease (AD). In the past years huge efforts have been dedicated to develop therapies and to investigate suitable biomarkers for *in vivo* imaging of Aβ plaques. Several therapies are in late stage clinical development and several positron emission tomography (PET) tracers to visualize Aβ plaques *in vivo* have been discovered.[1,2] On the other hand, there is emerging evidence that tau aggregates in the form of neurofibrillary tangles (NFT) or neuropil threads (NT) seem to be indicators of clinical symptoms of Alzheimer's disease.[3] Recently, a number of PET tracers targeting tau aggregates have been identified.[4–8] An internal program was initiated with the goal to investigate tau PET tracers with favorable selectivity versus Aβ plaques, high sensitivity and good PK properties.[9] This work resulted in 3 tracers, [^{11}C]RO6924963, [^{11}C]RO6931643, and [^{18}F]RO6958948 which were selected for a phase 1 clinical trial in healthy volunteers and AD patients. Part 1 of this trial was dedicated to the selection of the best tracers based on brain uptake, radiometabolites, signal to background ratio, and distribution. [^{18}F]RO6958948 exhibited the desired profile and was selected to enter part 2 of this trial which was focusing on test-retest variabilites and radiation dosimetry calculations. Overall, [^{18}F]RO6958948 showed favorable properties to visualize tau aggregates in human brains and might be a valuable biomarker for the development of therapies targeting NFTs and NTs in Alzheimer's disease (Fig. 1).

For the first preclinical *in vitro* and *in vivo* studies Medicinal Chemistry developed a four-step synthesis sequence to the nitroprecursor **1** of the tau PET tracer [^{18}F]RO6958948. This route included several chromatographic purifications and was suitable for mg-scale syntheses. To cope with an increasing demand of precursor which is required to supply clinical studies, an improved process was highly desirable. In this paper we wish to report the evaluation of different routes and the elaboration of a safe and scalable synthesis route towards precursor **1** (Fig. 2).

2. Results and discussion

2.1. Medicinal Chemistry approach to 1

The route for the preparation of **1** that was designed and executed by the Medicinal Chemistry team is outlined in Scheme 1.

Abbreviations: calcd, calculated; h, hour(s); IPC, in-process control; min, minutes; NFT, neurofibrillary tangle; NSB, non-specific binding; NT, neutropil thread; r.t., ambient (room) temperature.

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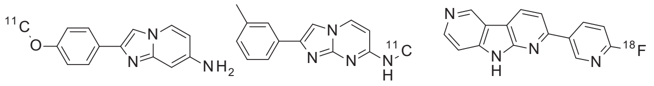

Fig. 1. Tau PET tracers tested in phase 1.

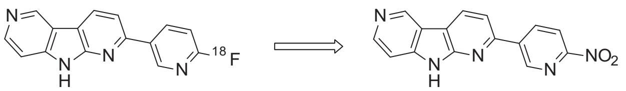

Fig. 2. Tracer precursor **1** used for the radiosynthesis of [^{18}F]RO6958948.

Central to this approach is the construction of the tricyclic diazacarbazole core via ring closure under relatively mild basic conditions. Starting from *tert*-butyl 3-iodopyridin-4-ylcarbamate and 2,6-dichloropyridin-3-ylboronic acid, bipyridyl intermediate **2** was formed using classical *Suzuki-Miyaura* type cross-coupling conditions. Purification can be accomplished by either normal phase silica gel chromatography or crystallization in order to obtain the product in good quality. Intramolecular cyclization of **2** to form 1,6-diazacarbazole **3** was attempted by modifying the procedure of Achab et al.[10] using equimolar amounts of 18-crown-6 and K_2CO_3 in DMF. The crown ether is facilitating the activation of the base via complexation and, thus, promoting the intramolecular nucleophilic substitution. However, these conditions also resulted in complete loss of the Boc protective group. Direct conversion of **3** to **1** under *Suzuki-Miyaura* conditions using the respective 2-nitropyridine-5-boronic acid pinacol ester and Pd(II)Cl_2(dppf) as catalyst failed[‡]. Re-protection of the pyrrole nitrogen to **4** was found to be advantageous and final *Suzuki-Miyaura* coupling gave the desired product **1** as mixture of **1** and Boc-protected **1** as side product. Separation of both products via preparative HPLC gave **1** in good purity yet low yield.[11]

An alternative access to **3** via the corresponding *N*-acetyl protected bipyridyl intermediate and subsequent intramolecular cyclization proved to be feasible but did not offer any advantages compared to the route outlined above.

Even though the original Medicinal Chemistry route (Scheme 1) represents a very short, four-step access to **1** and proved successful for the preparation of low mg-quantities, a number of points needed to be addressed in order to get a more robust and reproducible route for the preparation of multi-gram quantities of **1**. The involved starting materials are rather expensive and the protocols towards **1** are far from being robust which results in variable yields for each step. Chromatographic purifications were essential in 3 out of 4 steps. Finally, due to the loss of the Boc group during the intramolecular ring closure to **3** an additional protection step was necessary in order to get the final coupling reaction to proceed.

2.2. Process chemistry approaches to **1**

After selecting the development candidate, route optimization was initiated with the aim to find a scalable synthesis route to **1** which allows this compound to be produced on a multi-gram scale. Availability of starting materials, process safety, facile purifications, improved yields and overall cost of goods are other important aspects that were taken into account during route optimization.

Thus, our initial attempts focused on a) the evaluation of alternative access routes to intermediate **3** and b) finding alternative conditions and reagents for the final coupling step to **1**.

Our first approach started from either 2-chloro-6-methoxypyridin-3-amine or 2-chloro-6-methoxypyridine (Scheme 2). Diazotization of 2-chloro-6-methoxypyridin-3-amine with sodium nitrite and subsequent *Sandmeyer* reaction using CuCl/HCl gave the dichloropyridine intermediate **5**[12] after facile purification yet in slightly lower isolated yield than the chlorination of 2-chloro-6-methoxypyridine using *N*-chlorosuccinimide in acetonitrile. Even though 2-chloro-6-methoxypyridine was converted completely under the employed conditions, silica gel chromatography was indispensable to isolate the desired regioisomer from the obtained 4:1 mixture of 2,3- and 2,5-bis-chlorinated products. *Buchwald-Hartwig* amination with 4-aminopyridine using a Pd-BINAP catalyst system in refluxing toluene afforded bipyridylamine **6** in good yield (78%). The critical intramolecular ring closure towards diazacarbazole **7** was approached via Pd-catalyzed C—H activation and direct arylation using *tert*-butylphosphonium tetrafluoroborate and palladium acetate.[13,14] High temperature and dioxane as solvent were necessary to achieve a good conversion. Replacement of the methoxy group by chlorine was accomplished using standard chemistry. Cleavage of the methoxy group by aqueous HBr gave pyridone **8** which was subsequently converted to chloride **3** using $POCl_3$ in sulfolane.

Starting from crucial intermediate **3**, the final conversion to **1** was examined in more detail. As outlined above, the protection of the —NH group was necessary for applying *Suzuki-Miyaura* cross-coupling conditions with 2-nitropyridine-5-boronic acid pinacol ester. Another draw-back of this strategy was the mixture of protected and unprotected **1** obtained under these conditions. Performing this reaction in opposite direction was impossible as the transformation of **3** into its boronate ester by using tetramethyl-dioxoborolane dimer failed. Use of the corresponding potassium trifluoroborate instead of the boronate ester also failed. *Negishi*-type chemistry also seemed to be attractive. However, the transformation of 5-bromo-2-nitropyridine into its Zn-bromide did not work either in our hands.

Eventually, *Stille* cross-coupling conditions turned out to be the method of choice for the desired transformation. The necessary trimethylstannane reagent **9** could be prepared in moderate yield from 5-bromo-2-nitropyridine.[5,15] The subsequent coupling reaction with **3** in dioxane at 120 °C (closed reactor) resulted in up to 72% conversion to **1**. Preliminary purification using silica gel chromatography gave the crude product in 60% yield. Due to the low solubility of **1**, the final crystallization had to be done from hot DMSO which afforded **1** in good quality but poor yield (11% for this step).

Compared to the Medicinal Chemistry synthesis route, the number of steps increased yet the overall yield decreased. In addition, the presumably toxic tin reagent **9** used for the final coupling step needed to be prepared separately. On the other hand, the robustness of the route improved significantly. The final purification remained a challenge due to the low solubility of **1** in basically all common organic solvents.

Further evaluations of various steps resulted in a re-design of the route towards **1** while maintaining the overall synthesis strategy (Scheme 3). Bipyridylamine **10** was obtained via *Buchwald-Hartwig* amination from commercial 2-chloro-6-methoxypyridine and 4-amino-3-chloropyridine using a Pd/xanthphos catalyst system. Extraction and precipitation from methanol/water furnished **10** as white solid that was used directly in the next step. The direct arylation of **10** via Pd-catalyzed C—H activation using *tert*-butylphosphonium tetrafluoroborate, palladium acetate and potassium carbonate in dimethylacetamide at 135 °C remained the method of choice for the intramolecular cyclization to **7**. Silica

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[‡] A catalyst screening was not performed.