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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 nitro-precursor **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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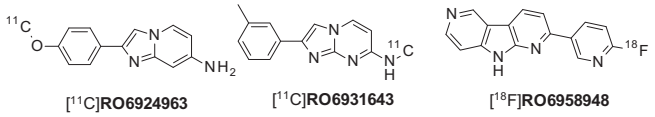


Fig. 1. Tau PET tracers tested in phase 1.

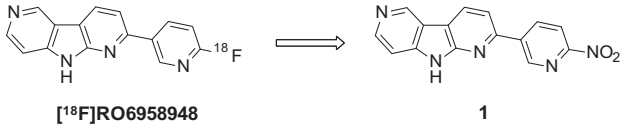


Fig. 2. Tracer precursor **1** used for the radiosynthesis of [^{18}F]RO6958948.

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.

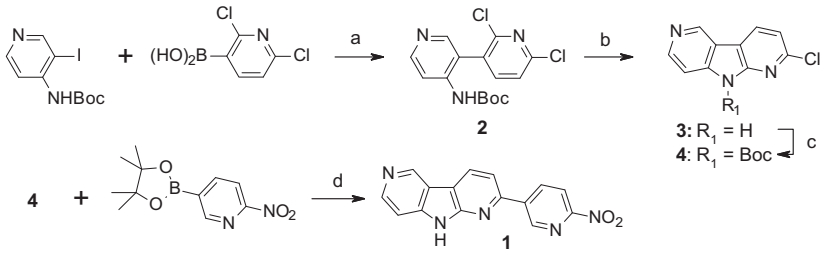
Starting from crucial intermediate **3**, the final conversion to **1** was examined in more detail. As outlined above, the protection of the —NH group was necessary for applying *Suzuki-Miyaura* cross-coupling conditions with 2-nitropyridine-5-boronic acid pinacol ester. Another draw-back of this strategy 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).

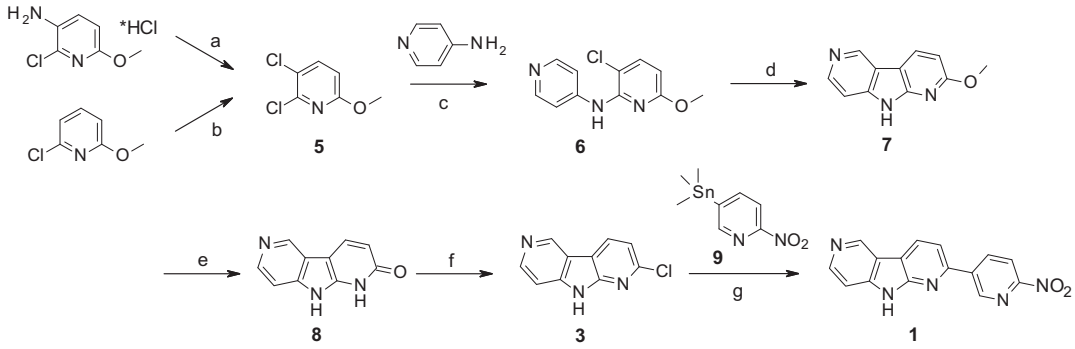
Compared to the Medicinal Chemistry synthesis route, the number of steps increased yet the overall yield decreased. In addition, the presumably toxic tin reagent **9** used for the final coupling step needed to be prepared separately. On the other hand, the robustness of the route improved significantly. 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

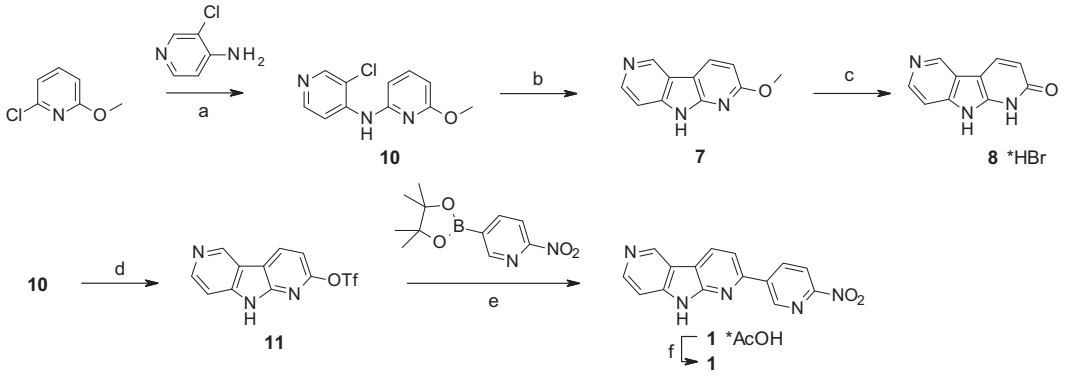
[‡] A catalyst screening was not performed.



Scheme 1. Medicinal Chemistry synthesis route to 9*H*-dipyrido[2,3-*b*;3′,4′-*d*]pyrrole **1**. *Reagents and conditions: a)* $Pd(OAc)_2$, PPh_3, Et_3N, DMF, 100 °C, 3 h, 63%; *b)* K_2CO_3, 18-crown-6, DMF, 100 °C, 3 h, 63%; *c)* NaH, DMF, 0 °C to rt, then Boc_2O, DMF, 0 °C to rt, 18 h, 73%; *d)* $[PdCl_2(dppf)]\cdot CH_2Cl_2$, K_2CO_3, DMF, 90 °C, 18 h, 13%.



Scheme 2. First Process Research synthesis route to tracer precursor **1**. *Reagents and conditions*: *a)* $NaNO_2$, CuCl, HCl/H_2O, 0 °C, then rt, 16 h, 60%; *b)* NCS, MeCN, 82 °C, 22 h, 70%; *c)* 4-aminopyridine, $Pd(OAc)_2$, BINAP, K_2CO_3, toluene, 110 °C, 20 h, 78%; *d)* $Pd(OAc)_2$, $(^tBu_3PH)BF_4$, NaO^tBu, 1,4-dioxane, 135 °C, 19 h, 81%; *e)* HBr, H_2O, 80 °C, 16 h, 82%; *f)* $POCl_3$, sulfolane, 100 °C, 22 h, 46%; g) $Pd(PPh_3)_4$, 17 h, 1,4-dioxane, 120 °C, 4 h, 11%.



Scheme 3. Final route to tracer precursor **1**. *Reagents and conditions*: *a)* $Pd(xantphos)Cl_2$, Cs_2CO_3, 1,4-dioxane, 102 °C, 72 h, 71%; *b)* $Pd(OAc)_2$, $(^tBu_3PH)BF_4$, K_2CO_3, DMA, 135 °C, 18 h, 80%; *c)* HBr, AcOH, 80 °C, 18 h, 80%; *d)* $C_6H_5N(SO_2CF_3)_2$, DIPEA, DMF, 20–25 °C, 2.5 h, then $KHCO_3$, H_2O, 50 °C, 18 h, 97%; *e)* $Pd(xantphos)Cl_2$, 1,4-dioxane, KF, 90 °C, 3.5 h, then AcOH, reflux, 27%; *f)* Et_3N, methanol, 60–65 °C, 1 h, 96%.

gel filtration and crystallization from *tert*-butyl methyl ether/*n*-heptane was necessary for the purification of this intermediate. Cleavage of the methoxy group using HBr in acetic acid at elevated temperature afforded pyridone **8** as its HBr salt, a white solid precipitating from acetic acid/ethyl acetate. Instead of converting pyridone **8** into chloride **3**, the hydroxy group was activated as triflate **11** employing bis(trifluoromethylsulfonyl)aniline under mild conditions. This final intermediate was isolated from DMF/water as light yellow solid. This activation and the use of potassium fluoride as base facilitated the final *Suzuki-Miyaura* cross-coupling reaction with 2-nitropyridine-5-boronic acid pinacol ester and Pd(xanthphos)Cl_2 as catalyst. Triflate **11** converted almost quantitatively to the desired tracer precursor **1**. What remained a challenge was the final purification of the product due to its intrinsic low solubility in organic solvents. The crude product was at first converted into its acetate salt after several crystallization-dissolution-filtration cycles and isolated in moderate yield. Finally, treatment with triethylamine in methanol provided the free base of the desired tracer precursor **1** almost quantitatively as yellow crystalline solid.

3. Conclusions

In summary, a safe and scalable synthesis route for the tau PET tracer precursor **1** devoid of any chromatographic purifications was developed. Key features of the route are three Pd-catalyzed reactions: *Buchwald-Hartwig* amination to bipyridylamine **10**, intramolecular ring closure to **7** via Pd-catalyzed C—H activation and finally *Suzuki-Miyaura* cross-coupling starting from triflate

11. The robustness of the process could be demonstrated on multigram scale and **1** could be prepared in five linear steps (11.4% overall yield) and high purity.

4. Experimental section

4.1. General methods

Unless otherwise noted, all reagents and chemicals were obtained from commercial suppliers and used without further purification. Non-aqueous reactions were carried out under an inert atmosphere of argon or nitrogen. Reactions and purifications were monitored using the following IPC methods: TLC (TLC plates F254, Merck), HPLC (Agilent 1260 Infinity with quaternary pump, ALS autosampler and DAD detector and InfinityLab Poroshell 120 EC-C_{18} 2.7 µm, 4.6 × 50 mm column using A = acetonitrile, B = water/acetonitril 95:5% v/v, C = 1‰ tetrabutylammonium hydrogen sulfate in acetonitrile/water 80:20% v/v at 25 °C at a flow of 1 mL/min with the following gradient: 0 min, 90% B; 1 min 90% B, 7 min, 10% B, 12 min 10% B, 13 min, 90% B and constantly 5% C), GC (Agilent 6850 with FID detector, integrated autosampler, Agilent HP-1 30 m x 0.32 mm x 0.25 µm column and the following gradient: 0 min, 60 °C; 2 min, 60 °C, 10 °C/min to 150 °C, 40 °C/min to 300 °C; 2 min, 300 °C) and LC–MS (Waters Acquity UPLC with binary pump, 2777C sample manager, column manager, PDA detector and SQD1 mass detector; Agilent Zorbax EclipsePlus C18 column) analysis. Flash column chromatography was carried out either using cartridges packed with silica gel (Isolute columns, Telos flash columns) on an ISCO machine or using glass columns packed with silica gel 60 (32–60 mesh, 60 Å). Preparative HPLC purification was carried out with a system consisting of Varian SD1 pumps, Gilson 215 Liquid Handler, Dionex UVD340U detector and SEDEX 75 ELS detector using a YMC Triart C_{18} 100 x 30mm column and a MeOH/water (0.1% formic acid) eluent system with 40 mL/min flow rate. The purity of isolated products was determined by HPLC (using the above described IPC method) or UPLC (Waters Acquity UPLC, Acquity BEH C_{18} 1.7 µm, 2.1 x 50 mm column at 35 °C using A = 0.1% v/v formic acid in water and B = 0.1% v/v formic acid in acetonitrile and a flow rate of 0.7 mL/min with the following gradient: 0 min, 5% B; 0.1 min 5% B; 5.0 min, 60% B; 5.9 min 60% B; 5.91 min, 5% B; 8.0 min, 5% B) . High-resolution LC-MS spectra were recorded with an Agilent LC system consisting of an Agilent 1290 high-pressure system, a CTC PAL autosampler, and an Agilent 6520 QTOF. The separation was achieved on a Zorbax EclipsePlus C_{18} 1.7 µm, 2.1 x 50 mm column at 55 °C (A = 0.01% v/v formic acid in water; B = 0.01% v/v formic acid in acetonitrile) at a flow of 1 mL/min with the following gradient: 0 min, 5% B; 0.3 min, 5% B; 4.5 min, 99% B; 5 min, 99% B. ^{1}H NMR spectra were measured on a Bruker 300 MHz instrument, a Bruker 600 MHz instrument, or a Bruker Avance 400 MHz instrument. ^{13}C NMR spectra were measured at 151 MHz on a Bruker 600 MHz instrument in a 5 mm TCI cryoprobe at 298 K. A TMS internal standard was used for experiments done in $CDCl_3$. The deuterated DMSO d_6 solvent signal was used as the reference with 2.50 ppm.

4.2. Synthesis procedures

*tert-Butyl N-[3-(2,6-dichloro-3-pyridyl)-4-pyridyl]carbamate (**2**).* A pre-heated flask under argon was charged with *tert*-butyl 3-iodopyridin-4-ylcarbamate (4.56 g, 14.2 mmol), 2,6-dichloropyridin-3-ylboronic acid (5.46 g, 28.4 mmol), palladium (II) acetate (320 mg, 1.42 mmol) and triphenylphosphine (371 mg, 1.41 mmol). Triethylamine (4.32 g, 5.94 mL, 42.7 mmol) in DMF (137 mL) was added and the reaction mixture was stirred at 100 °C for 3 h. The solvent was evaporated almost completely. Water was added and the crude product suspension was extracted twice with ethyl acetate. The combined organic layers were washed with water (3×), dried over anhydrous Na_2SO_4 and the solvent was evaporated. Trituration of the crude product with dichloromethane afforded 1.92 g of the desired product. The dichloromethane phase was evaporated and purified by flash chromatography (SiO_2; ethyl acetate to *n*-heptane gradient) to yield in total 3.39 g (~90% purity, 63% yield) of **2** as light yellow solid. This material was used as such in the following step. ^{1}H NMR (300 MHz, DMSO d_6) δ 1.42 (s, 9H), 7.66 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 5.6 Hz, 1H), 7.88 (d, J = 8.1 Hz, 1H), 8.28 (s, 1H), 8.48 (d, J = 5.8 Hz, 1H), 9.08 (s, 1H); LC-HRMS (m/z): $[M+H]^+$ calcd for $C_{15}H_{15}Cl_2N_3O_2+H^+$: 340.0621, found: 340.0624.

*2-Chloro-9H-dipyrido[2,3-b;3′,4′-d]pyrrole (**3**). Method A (from **2**).* A suspension of *tert*-butyl *N*-[3-(2,6-dichloro-3-pyridyl)-4-pyridyl]carbamate **2** (264 mg, 776 µmol), K_2CO_3 (215 mg, 1.55 mmol) and 18-crown-6 (226 mg, 854 µmol) in DMF (15.8 mL) was stirred at 100 °C under argon for 3 h. water was added and the product was extracted twice with ethyl acetate. The combined organic layers were washed twice with water and brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated. Trituration of the crude product with a small amount of MeOH and drying afforded **3** (105 mg, 63%) as light yellow solid. *Method B (from **8**):* A suspension of 2-oxo-2,9-dihydro-1*H*-pyrrolo[2,3-*b*:4,5-*c*′]dipyridine **8** (1.0 g, 5.4 mmol) was suspended in sulfolane (20 mL). Phosphorus oxychloride (5.0 mL, 53.6 mmol) was added and the mixture was stirred at 100 °C for 22 h. After cooling down to r.t. the reaction mixture was diluted with water (80 mL) and neutralized with Na_2CO_3 until pH 5–7 was reached. The reaction mixture was further diluted with water (50 mL) and extracted with ethyl acetate/THF (1:1) twice. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was suspended in isopropanol/water (1:3, 100 mL). The slowly forming light-yellow precipitate was filtered off, washed with water and dried *in vacuo* to obtain 510 mg (46%) of **3**. ^{1}H NMR (300 MHz, DMSO d_6) δ 7.41 (d, J = 8.1 Hz, 1H), 7.51 (d, J = 5.5 Hz, 1H), 8.52 (d, J = 5.6 Hz, 1H), 8.68 (d, J = 8.1 Hz, 1H), 9.39 (s, 1H), 12.47 (br s, 1H); LC-HRMS (m/z): $[M+H]^+$ calcd for $C_{10}H_6ClN_3+H^+$: 204.0323, found: 204.0333.

*2-Chloro-dipyrido[2,3-b;3′,4′-d]pyrrole-9-carboxylic acid tert-butyl ester (**4**).* A suspension of NaH (60%, 26.5 mg, 607 µmol) in dry DMF (1.5 mL) was cooled under argon to 0 °C and a solution of 2-chloro-9*H*-dipyrido[2,3-*b*;3′,4′-*d*]pyrrole **3** (103 mg, 506 µmol) in dry DMF (3.0 mL) was added. Stirring was continued at 0 °C for 10 min, then at rt for 30 min. After cooling down to 0 °C and addition of di-*tert*-butyl dicarbonate (132 mg, 141 µL, 1.05 mmol) in dry DMF (0.75 mL), stirring was continued at r.t. overnight. Water was added and the reaction mixture was extracted twice with ethyl acetate. The combined organic layers were washed twice with water and brine, dried over anhydrous Na_2SO_4, filtered and evaporated. **4** was obtained after purification by silica gel chromatography (dichloromethane:MeOH gradient) and drying *in vacuo* as off-white solid (113 mg, 73%). ^{1}H NMR (300 MHz, DMSO d_6) δ 1.70 (s, 9H), 7.64 (d, J = 8.1 Hz, 1H), 8.11 (dd, J = 0.9, 5.8 Hz, 1H), 8.69 (d, J = 5.7 Hz, 1H), 8.76 (d, J = 8.1 Hz, 1H), 9.48 (d, J = 0.8 Hz, 1H); LC-HRMS (m/z): $[M+H]^+$ calcd for $C_{15}H_{14}ClN_3O_2+H^+$: 304.0854, found: 304.0851.

*2,3-Dichloro-6-methoxypyridine (**5**).* A solution of 3-amino-2-chloro-6-methoxypyridine hydrochloride (6.2 g, 31.6 mmol) in concentrated hydrochloric acid (100 mL) was cooled down to −5 °C. At this temperature a solution of $NaNO_2$ (10.9 g, 158 mmol) in water (50 mL) was added within 30 min. After stirring for 15 min, CuCl (31.3 g, 316 mmol) was added slowly within 30 min. Stirring was continued at r.t. for 16 h and ammonia (25% aqueous solution, 150 mL) was slowly added at 10 °C. The reaction mixture was extracted with *tert*-butyl methyl ether (3 × 50 mL) and the

organic phases were dried and evaporated. The crude product was dissolved in *n*-heptane (50 mL), charged on silica gel (25 g) and eluted with *n*-heptane (250 mL) under reduced pressure. The filtrate was evaporated and the solid was dried *in vacuo* to obtain **5** as white solid (3.4 g, 60%). ^{1}H NMR (400 MHz, DMSO d_6) δ 3.93 (s, 3H), 6.65 (d, J = 8.6 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H); LC-MS (m/z): $[M+H]^+$ for $C_6H_5Cl_2NO+H^+$: 178.0.

3-Chloro-6-methoxy-N-(4-pyridyl)pyridin-2-amine (***6***). Under argon atmosphere, palladium acetate (110 mg, 0.49 mmol) and 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP; 310 mg, 0.5 mmol) were suspended in toluene (8 mL) and stirred at 100 °C for 15 min. The reaction mixture was allowed to cool down to r.t. and 2,3-dichloro-6-methoxypyridine **5** (1.8 g, 10.1 mmol), 4-aminopyridine (1.25 g, 13.3 mmol), K_2CO_3 (5 g, 36.2 mmol) and toluene (28 mL) were added consecutively and the reaction mixture was stirred at 110 °C for 20 h. After cooling down to ambient temperature the reaction mixture was filtrated and the residue was rinsed with toluene (20 mL). The clear filtrate was evaporated to a small volume (10 mL) and purified by silica gel chromatography using an ethyl acetate/*n*-heptane gradient (80:20 to 100:0 (v/v)). Evaporation of the product fractions and drying *in vacuo* afforded **6** as white solid (1.95 g, 78%). ^{1}H NMR (400 MHz, DMSO d_6) δ 3.97 (s, 3H), 6.32 (d, J = 8.6 Hz, 1 H), 7.08 (br s, 1H), 7.51 (d, J = 8.6 Hz, 1H), 7.58–7.63 (m, 2H), 8.42–8.49 (m, 2H); LC-MS (m/z): $[M+H]^+$ for $C_{11}H_{10}ClN_3O+H^+$: 236.1.

2-Methoxy-9H-pyrrolo[2,3-b:4,5-c′]dipyridine (***7***). A 4.5 L glas reactor was purged with argon and charged with palladium (II) acetate (5.15 g, 23 mmol), tri-*tert*-butylphosphonium tetrafluoroborate (7.73 g, 26.6 mmol) and dimethylacetamide (200 mL). This mixture was stirred at r.t. for 15 min. *N*-(3-chloropyridin-4-yl)-6-methoxypyridin-2-amine **10** (100.0 g, 424 mmol), K_2CO_3 (120 g, 870 mmol) and dimethylacetamide (800 mL) were added and the reaction mixture was heated under vigorous stirring to 135 °C for 16 h. The dark mixture was cooled down to r.t., diluted with ethyl acetate/ethanol 9:1 (1 L), filtrated and rinsed with little ethyl acetate. The filtrate was evaporated under reduced pressure and the residue was dried *in vacuo* and re-dissolved in 1,4-dioxane (1.5 L) and ethyl acetate/methanol 8:2 (1.5 L) at 75 °C. The green suspension was cooled down to r.t., charged to conditioned silica gel (300 g) and eluted under slightly reduced pressure with 1,4-dioxane/ethyl acetate/methanol (50:45:5, 1.2 L). The clear filtrate was evaporated under reduced pressure and the residue was dissolved in *tert*-butyl methyl ether (300 mL). *n*-Heptane (300 mL) was slowly added within 45 min and the obtained suspension was stirred at r.t. for 75 min. The solid was filtered off, washed with *n*-heptane and dried *in vacuo* to obtain **7** as greenish solid (72.3 g, 80%; GC: 94.1% a/a). ^{1}H NMR (600 MHz, DMSO d_6) δ 3.95 (s, 3H), 6.74 (d, J = 8.5 Hz, 1H), 7.41 (dd, J = 1.0, 5.6 Hz, 1H), 8.39 (d, J = 5.6 Hz, 1H), 8.48 (d, J = 8.5 Hz, 1H), 9.23 (d, J = 0.9 Hz, 1H), 12.21 (br s, 1H); ^{13}C NMR (151 MHz, DMSO d_6) δ 53.4, 103.6, 106.5, 106.6, 118.0, 132.2, 141.8, 142.1, 143.7, 150.3, 163.2; LC-HRMS (m/z): $[M+H]^+$ calcd for $C_{11}H_9N_3O+H^+$: 200.0826, found: 200.0840.

1,9-Dihydro-dipyrido[2,3-b;3′,4′-d]pyrrol-2-one hydrobromide (***8***). A 4.5 L glas reactor was purged with argon and charged with 2-methoxy-9*H*-pyrrolo[2,3-*b*:4,5-*c*′]dipyridine (**7**) (72 g, 361 mmol). Acetic acid (575 mL) was added and the mixture was treated with HBr (33 wt% in AcOH, 290 mL, 1.66 mol). The milky suspension was stirred at 80 °C for 16 h. The reaction mixture was cooled down to r.t. and ethyl acetate (1.3 L) was added within 20 min. Stirring was continued for 15 min and the solid was filtered off. The residue was washed with ethyl acetate (300 mL) and *n*-heptane (200 mL) and dried *in vacuo* to obtain **8** as HBr salt (white solid; 100.3 g, 80%). This material was used as such in the following step. ^{1}H NMR (400 MHz, DMSO d_6) δ 6.78 (d, J = 8.6 Hz, 1H), 7.94 (d, J = 6.4 Hz, 1H), 8.62 (d, J = 6.4 Hz, 1H), 8.63 (d, J = 8.3 Hz, 1H), 9.62 (s, 1H), 13.28 (s, 1H), 14.80 (br s, 1H); LC-HRMS (m/z): $[M+H]^+$ calcd for $C_{10}H_7N_3O+H^+$ (free base): 186.0669, found: 186.0672.

Trimethyl-(6-nitro-3-pyridyl)stannane (***9***). 5-Bromo-2-nitropyridine (4 g, 19.7 mmol) was dissolved in 1,4-dioxane (48.0 mL) and tetrakis(triphenylphosphine)palladium(0) (800 mg, 692 μmol) was added, followed by hexamethylditin (9.48 g, 6.00 mL, 28.9 mmol). The mixture was stirred until conversion was complete for 1.5 h at 100 °C. The mixture was concentrated and dried *in vacuo* (5 mbar) at 55 °C. The residue was dissolved in ethyl acetate (80 mL) and *n*-heptane (320 mL) and silica gel (20 g) was added. The resulting slurry was stirred for 10 min at r.t., filtered over a plug of silica gel (20 g, pretreated with *n*-heptane) and washed with ethyl acetate/*n*-heptane 1:4 (v/v, 100 mL). The filtrate was concentrated *in vacuo* (5 mbar) at 55 °C. The brown residue was triturated with *n*-heptane (10 mL), filtered and the precipitate was washed with *n*-heptane and dried *in vacuo* (5 mbar) at 55 °C to yield 3.01 g (53%) of **9** as brown crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.43 (s, 9H), 8.14 (dd, J = 1.6 Hz, 7.8 Hz, 1H), 8.18 (dd, J = 0.8 Hz, 7.8 Hz, 1H), 8.63–8.70 (m, 1H); LC-MS (m/z): $[M+H]^+$ for $C_8H_{12}N_2O_2Sn+H^+$: 289.0.

N-(3-Chloropyridin-4-yl)-6-methoxypyridin-2-amine (***10***). A 4.5 L glas reactor was purged with argon and charged with 1,4-dioxane (1.8 L). 2-Chloro-6-methoxypyridine (80.0 g, 557 mmol), 3-chloropyridin-4-amine (88 g, 685 mmol), Cs_2CO_3 (252 g, 775 mmol) and acetic acid (3.2 mL, 56 mmol) were consecutively added. The suspension was stirred at r.t. for 20 min. Dichloro[9,9-*dimethyl*-4,5-*bis*(*diphenyl-phosphino*)*xanthene*]palladium(II) (22.1 g, 29.2 mmol) in 1,4-dioxane (210 mL) was added and the reaction mixture was stirred at 100 °C for 4 h. After cooling down to r.t. toluene (2.0 L) was added. The suspension was filtered off and the solid was washed with toluene (300 mL). The filtrate was evaporated under reduced pressure and the obtained yellow solid was dissolved in methanol (1.2 L) at r.t.. Water (800 mL) was slowly added within 75 min and the slurry was stirred at r.t. for 15 min and subsequently at 0–5 °C overnight. The crystalline product was filtered off, washed with a methanol/water mixture (1:2, 600 mL) and subsequently dried *in vacuo* to obtain **10** as white solid (111 g, 71%; GC: 97.9% a/a). ^{1}H NMR (400 MHz, DMSO d_6) δ 3.85 (s, 3H), 6.41 (dd, J = 0.7, 8.1 Hz, 1H), 6.91 (dd, J = 0.7, 7.8 Hz, 1H), 7.64 (t, J = 7.9 Hz, 1H), 8.29 (d, J = 5.6 Hz, 1H), 8.36 (d, J = 5.6 Hz, 1H), 8.45 (s, 1H), 8.76 (br s, 1H); ^{13}C NMR (151 MHz, DMSO d_6) δ 53.8, 102.7, 105.4, 113.6, 119.0, 141.2, 144.5, 148.6, 149.4, 152.7, 162.7; LC-HRMS (m/z): $[M+H]^+$ calcd for $C_{11}H_{10}ClN_3O+H^+$: 236.0592, found: 236.0608.

9H-Pyrrolo[2,3-b:4,5-c′]dipyridin-2-yl trifluoromethanesulfonate (***11***). A 4.5 L glas reactor was purged with argon and charged with DMF (800 mL). 1,9-Dihydro-dipyrido[2,3-*b*;3′,4′-*d*]pyrrol-2-one hydrobromide **8** (80.0 g, 231 mmol) and N,*N*-diisopropylethylamine (175 mL, 1.0 mol) were consecutively added. The mixture was cooled to 5 °C and treated with *N*,*N*-bis(trifluoromethylsulfonyl)aniline (125 g, 350 mmol) and DMF (100 mL). After warming up to r.t. the reaction mixture was stirred for 1 h and another portion of *N*,*N*-bis(trifluoromethylsulfonyl)aniline (42 g, 118 mmol) and DMF (100 mL) was added. Stirring was continued at r.t. for another 3.5 h. The orange suspension was treated with $KHCO_3$ (120 g, 1.2 mol) and water (400 mL) and stirred at 50 °C for 18 h. Water (1.0 L) was added slowly and the mixture was cooled to 0–5 °C and stirred at that temperature for 90 min. The precipitate was filtered off, washed with water (500 mL) and *n*-heptane (200 mL) and dried *in vacuo* to obtain **11** as light-yellow solid (73.3 g, 97%; HPLC (220 nm): 96.9% a/a). ^{1}H NMR (400 MHz, DMSO d_6) δ 7.46 (d, J = 8.1 Hz, 1H), 7.55 (dd, J = 1.1, 5.6 Hz, 1H), 8.57 (d, J = 5.6 Hz, 1H), 8.95 (d, J = 8.3 Hz, 1H), 9.47 (d, J = 1.0 Hz, 1H), 12.83 (br s, 1H); ^{13}C NMR (151 MHz, DMSO d_6) δ 107.0, 107.2, 114.6, 116.7, 118.2 (q, J_{CF} = 320 Hz), 134.4, 143.8, 144.0,

145.6, 149.5, 153.0; LC-HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{11}H_6F_3N_3O_3S+H^+$: 318.0162, found: 318.0166.

*2-(6-Nitro-pyridin-3-yl)-9H-dipyrido[2,3-b;3′,4′-d]pyrrole (**1**). Method A (from **4**):* 2-Chloro-dipyrido[2,3-*b*;3′,4′-*d*]pyrrole-9-carboxylic acid *tert*-butyl ester **4** (285 mg, 938 μmol), 2-nitropyridine-5-boronic acid pinacol ester (469 mg, 1.88 mmol), [1,1′-bis (diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane complex (34.5 mg, 42.2 μmol) and K_2CO_3 (389 mg, 2.81 mmol) were combined under argon in a reaction flask. DMF (24 mL) was added and the flask was sealed. The reaction mixture was stirred at 90 °C for 18 h. Filtration through Celite® and subsequently through a small pad of silica gel (neutral, 60 Å, mesh 32–63) was followed by rinsing with DMF and evaporation to dryness. The brown solid was dissolved in DMF (20 mL) and DMSO was added until a clear solution resulted. After filtration, the solvents were evaporated to almost dryness. Purification by preparative HPLC (YMC Triart C18 100 × 30 mm–5 μm; water with 0.1% Et_3N/MeCN gradient) provided **1** as yellow solid (37 mg, 13%). *Method B (from **3**):* 2-Chloro-9*H*-dipyrido[2,3-*b*;3′,4′-*d*]pyrrole (**3**) (500 mg, 2.46 mmol) was dissolved in 1,4-dioxane (25.0 mL) and 2-nitro-5-(trimethylstannyl)pyridine **9** (1.00 g, 3.49 mmol) and tetrakis(triphenylphosphine)palladium(0) (1.00 g, 865 μmol) were consecutively added under an argon atmosphere in a pressure vial. The vial was capped and the mixture was stirred at 120 °C for 4 h. After that time, the conversion was ca. 65% by HPLC. After cooling to r.t., the mixture was diluted with ethyl acetate (50 mL) and silica gel (10 g) was added. The resulting slurry was concentrated *in vacuo* and dried at 55 °C and 20 mbar. The residue was directly subjected to a column chromatography using silica gel (50 g) and an ethyl acetate/methanol (100:0 to 92:8 v/v) gradient. The product containing fractions were combined and concentrated *in vacuo* to obtain the crude product as a yellow solid (460 mg). The crude products of 4 identical experiments were pooled and dissolved in ethyl acetate/methanol 9:1 (v/v, 800 mL) at 60 °C. Silica gel (20 g) was added and the resulting mixture was concentrated to a volume of ca. 200 mL. 1,4-Dioxane (100 mL) was added and the slurry was concentrated and dried *in vacuo* at 55 °C and 5 mbar. The resulting yellow powder was directly purified by column chromatography using silica gel (100 g) and eluting with a gradient of ethyl acetate/methanol (98:2–92:8 v/v, 1.5 L) and 1,4-dioxane (500 mL). The product fractions were combined and concentrated *in vacuo* to yield 1.04 g of purified product. This material was dissolved in DMSO (18 mL) at 150 °C and after cooling to r.t. the solution was stirred for 4 h. The resulting suspension was filtered, the crystals were washed with ethyl acetate (5 mL) and dried *in vacuo* (5 mbar) at 55 °C to afford 360 mg of a yellow crystalline powder. The powder was suspended in water (22 mL) at 80 °C and stirred for 15 min at that temperature, followed by 1 h at r.t.. The suspension was filtered, the crystals were washed with water and dried *in vacuo* (5 mbar) at 60 °C to afford **1** as a yellow crystalline powder (310 mg, 11% yield). *Method C (from **11**): a)* A 4.5 L glas reactor was purged with argon and consecutively charged with 9*H*-pyrrolo[2,3-*b*:4,5-*c*′]dipyridin-2-yl trifluoromethanesulfonate **11** (57.2 g, 180 mmol), 2-nitro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (86.2 g, 345 mmol), potassium fluoride (114 g, 1.96 mol), 1,4-dioxane (1.40 L) and water (570 mL) and was stirred at r.t. for 15 min. Then dichloro[9,9-*dimethyl*-4,5-*bis* (*diphenylphosphino*)-*xanthene*]palladium(II) (22.8 g, 30.2 mmol) in 1,4-dioxane (280 mL) was added and the mixture was stirred at 90 °C for 4.5 h. After cooling to r.t., the reaction mixture was treated with ethanol (1.7 L) and stirred at 0–5 °C for 4 h. The precipitate was filtered off, washed with ethyl acetate and sucked to dryness. The wet dark-brown solid was dissolved in refluxing acetic acid, hot filtrated under vacuum and washed with hot acetic acid. The clear solution was warmed up to 80 °C, cooled to r.t. and stirred for 20 h. The crystals were filtered off and washed with acetic acid/ethyl acetate 1:1 (200 mL), ethyl acetate (500 mL) and *n*-pentane (500 mL) and dried *in vacuo* to obtain a yellow-brownish crude product (HPLC (260 nm): 99.6% a/a, contains inorganic impurities). The crude acetate (27 g) was suspended in acetic acid (540 mL) and water (27 mL) and heated to reflux. The hot and turbid solution was hot filtrated and the brown residue was washed with acetic acid. The filtrate was warmed up until getting clear, cooled to r.t., stirred for 18 h and filtrated. The crystalline residue was washed with acetic acid/ethyl acetate 1:1, ethyl acetate and *n*-pentane and dried *in vacuo* to obtain 19.6 g of a dark yellow crude product (HPLC (260 nm): 99.5% a/a) which was suspended in acetic acid (390 mL) and water (20 mL) and heated to reflux. The clear solution was cooled to 80–90 °C and charged with charcoal (5 g). After heating to reflux, the suspension was filtrated and the residue was washed with acetic acid. The clear filtrate was partly evaporated and ethyl acetate was added to the resulting slurry. After cooling to r.t. the slurry was filtered off and the crystals were washed with ethyl acetate and *n*-pentane and dried *in vacuo* to obtain **1** as acetate salt (yellow crystalline powder; 17.2 g, 27%; HPLC (260 nm): 99.8% a/a). *b)* A 500 mL flask was purged with argon and charged with 2-(6-nitropyridin-3-yl)-9H-pyrrolo [2,3-*b*:4,5-*c*′]dipyridine acetate (15.50 g, 44.1 mmol). Methanol (310 mL) and triethylamine (9.5 mL, 68.2 mmol) were added and the reaction mixture was heated to 60–65 °C for 1 h. After cooling to r.t. stirring was continued for another 2 h. The suspension was filtrated and the yellow solid was washed with little methanol, dried, treated with water (60 mL) and ultrasonicated until a homogeneous suspension was obtained. All volatiles were removed and the product was obtained after drying *in vacuo* (5 mbar) at 60 °C as yellow solid (12.5 g, 96%; UPLC (263 nm): 99.2% a/a). ^{1}H NMR (400 MHz, DMSO d_6) δ 7.52 (dd, J = 1.1, 5.6 Hz, 1H), 8.20 (d, J = 8.3 Hz, 1H), 8.49 (d, J = 8.6 Hz, 1H), 8.54 (d, J = 5.6 Hz, 1H), 8.84 (d, J = 8.1 Hz, 1H), 8.95 (dd, J = 2.1, 8.6 Hz, 1H), 9.43–9.47 (m, 2H), 12.53 (s, 1H); ^{13}C NMR (151 MHz, DMSO d_6) δ 106.8, 114.4, 114.8, 117.2, 118.6, 130.6, 138.2, 139.9, 144.0, 144.1, 145.8, 147.1, 149.1, 152.1, 156.2; LC-HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{15}H_9N_5O_2+H^+$: 292.0829, found: 292.0827.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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