# Synthesis and biodistribution of [$^{131}$I]IMPY

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**Abstract** The synthesis and biodistribution of β-amyloid plaques imaging agent [$^{131}$I]-2- (4′-dimethylaminophenyl)-6-iodoimidazo[1,2-α] pyridine ([$^{131}$I]IMPY) were reported. The chemical structure of the labeling precursor 2-(4′-dimethylaminophenyl)-6- (tributylstannyl) imidazo[1，2-α] pyridine and all its intermediates were verified by IR,HNMR and MS. The radioiodinated compound was prepared using iododestannylation reaction by hydrogen peroxide. Final radiochemical purity was above 95% determined by TLC. The in vivo biodistribution of [$^{131}$I]IMPY in normal mice showed excellent brain uptake and washout, indicating this thioflavin-T based small molecular probe has potential for in vivo imaging amyloid deposits.

**Keywords** β-amyloid plaques, [$^{131}$I]IMPY, Synthesis

**CLC number** R817

## 1 Introduction

Alzheimer's disease (AD), a major public health problem, will cause an increasing enormous financial and emotional cost on society as the population lives in the next 20 years. Formation of β-amyloid (Aβ) plaques in the brain is a pivotal event in the pathology of AD. The accumulation of Aβ plaques is now considered one of the most significant factors in AD. Aβ aggregate-specific probes for in vitro and in vivo studies of Aβ plaques are potentially important for diagnosis and monitoring of therapeutic effects of drugs aiming at lowering the Aβ burden in the brain by noninvasive imaging[1-3].

Systematic preliminary studies showed that 2-(4′-dimethylaminophenyl) -6-iodoimidazo [1, 2-α] pyridine (IMPY) displayed high binding affinity for Aβ aggregates ($K_i$=(15±5)nmol/L) and selective amyloid plaque labeling in post mortem AD brain sections. The in vivo brain uptake showed that the initial uptake of [$^{125}$I]IMPY in normal mice was sufficiently high for potential human studies. IMPY exhibited a rapid brain clearance from normal mouse brain. Ex vivo labeling of amyloid plaques in Tg2576 transgenic mice showed selective retention of radioactivity in Tg mouse brain relative to aged-matched control litter mates. The plaques labeled by [$^{125}$I]IMPY were found to be identical to those stained with Thioflavin-S. These promising results suggested that IMPY might be a good candidate as a SPECT imaging agent for amyloid plaque in patients with Alzheimer's disease[4-7].

In this paper, we reported the synthesis and biodistribution study of $^{131}$I-IMPY. Zhuang Zhi-Ping et al[6] reported that 2-(4'-(N,N-dimethyl) aminophenyl)-6-bromoimidazo[1,2-α]pyridine (Ⅲ) was prepared by the reaction of 2-bromo-4'- dimethylaminoacetophenone and 2-amino-5-bromopyridine. Considering that 2-bromo-4'-dimethylaminoacetophenone was difficult to prepare and not commercially available, we coupled 2-amino-5-bromopyridine (commercially available) and 2-bromo-4'-nitro- acetophenone (commercially available) to afford 2-(4'-nitrophenyl)-6-bromoimidazo[1,2-α]pyridine (Ⅰ) and the total yield of 2-(4'-(N,N-dimethyl)aminophenyl) -6-(tri-n-butyltin) imidazo [1,2-α] pyridine (Ⅳ) was 28% and it was higher than that reported in literature (8.8%)[6].

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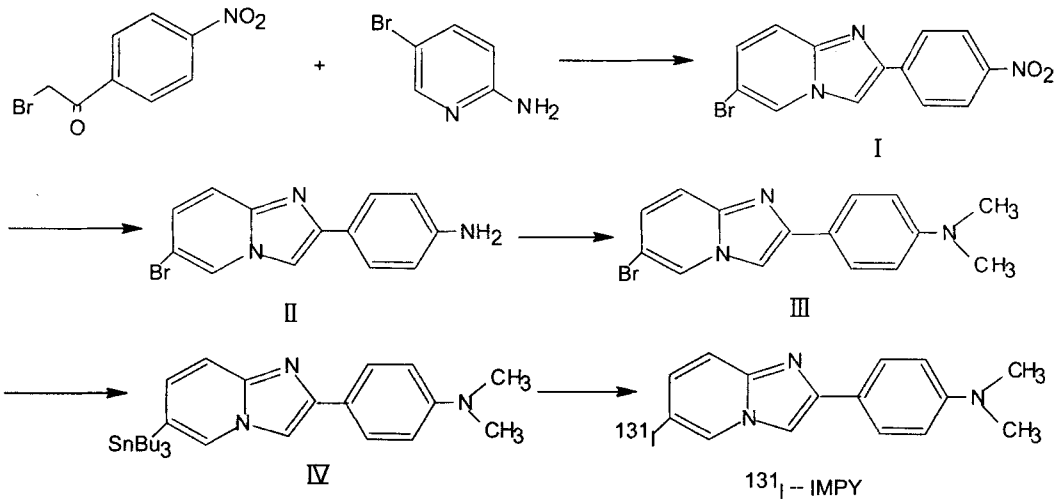
## 2 Materials and methods

### 2.1 General

All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. When reactions were worked up by extraction with chloroform ($CHCl_3$), organic solutions were dried with anhydrous $Na_2SO_4$ and concentrated with a rotary evaporator under reduced pressure.

Melting points were determined on Yanadimoto apparatus and uncorrected. Column chromatography was performed using silica gel, 100—200 mesh. $^1$HNMR spectra were recorded on an AM spectrometer at 400 MHz, with $CDCl_3$ as solvent and tetramethylsilane (TMS) as the internal standard (0 μg/g). Mass spectra were run on Varian MAT 2.2 spectrometer.

### 2.2 Synthesis of [$^{131}$I]IMPY




**2.2.1** 2-(4'-nitrophenyl)-6-bromoimidazo[1,2-α] pyridine (Ⅰ)[8]

A mixture of 2-amino-5-bromopyridine(3.5g) and 2-bromo-4'-nitroacetophenone (2.4g) in acetone (50mL) was refluxed for 3h.The material which precipitated was collected and the volume of the mother liquor was reduced by rotary evaporation and a second crop was collected. Normally the initial product was the hydrate of the desired product and aromatization was effected by dissolving it in 50 mL enthanol containing a little hydrogen bromide and refluxing for 1h, cooled to room temperature. The mixture was adjusted to pH 7 with 1 mol/L sodium hydroxide. The crude product was precipitated and was collected by filtration. The crude product was crystallized form pyridine to provide compound Ⅰ (3.0g, 95.5%) as yellow needle crystallite, mp>250℃. IR ($cm^{-1}$)：3086, 1639, 1599, 1512, 1337；MS:376 (M+2Na), 320, 318(M+1); $^1$HNMR: δ7.42 (dd,1H), 7.61 (d,1H), 8.22 (m,2H), 8.29 (m,2H), 8.56 (s,1H), 8.92 (m,1H).

**2.2.2** 2-(4'-aminophenyl)-6-bromoimidazo[1,2-α] pyridine(Ⅱ)

Compound Ⅰ (3.0g) and stannous chloride dehydrate (9.0g) were added to a solution of 50 mL enthanol and 20 mL of concentrated hydrochloric acid at 0℃. The mixture was stirred at room temperature overnight. The mixture was adjusted to pH 10 with 12 mol/L sodium hydroxide and extracted with chloroform (50mL×4). The combined organic layers were washed once with $H_2O$ (150mL), dried and concentrated under reduced pressure to give compound Ⅱ (2.2g, 81.2%) as yellow solid, mp>250℃. IR ($cm^{-1}$)：3318, 3194, 1644,1610, 1486; MS:313, 311 (M+Na), 291, 289(M+1), 211 (M-Br); $^1$HNMR: δ3.78 (s,2H), 6.76 (d,2H), 7.18 (dd,1H), 7.48 (d,1H), 7.68 (s,1H), 7.74 (d,2H), 8.22 (m,1H).

**2.2.3** 2-(4'-(N,N-dimethyl)aminophenyl)-6-bromoimidazo[1,2-α]pyridine(Ⅲ)

A mixture of compound Ⅱ (1.0g), $K_2CO_3$ (5.0g) and iodomethane (2.0mL) in 30mL DMF was stirred at 100℃ for 2h. The mixture was cooled to room temperature and extracted with chloroform (100mL×

3). The combined organic layers were washed once with $H_2O$ (200mL), dried and concentrated under reduced pressure. Column chromatography of the crude product on silica gel and elution with EtOAc afforded compound Ⅲ(0.5g, 45.9%) as little yellow solid, mp 233~235℃, TLC (VHex/VetOAc=1:1) $R_f$=0.81. IR ($cm^{-1}$): 3026, 2922, 2801, 1613, 1553, 1489; MS:341, 339 (M+Na), 319, 317 (M+1), 239 (M-Br); $^1$HNMR: δ3.00 (s,6H), 6.78 (d,2H), 7.18 (dd,1H), 7.48 (d, 1H), 7.69 (s,1H), 7.81 (d,2H), 8.22 (m,1H).

**2.2.4** 2-(4'-(N,N-dimethyl)aminophenyl)-6-(tri-n-butyltin) imidazo[1,2-α] pyridine (Ⅳ)

To a solution of compound Ⅲ (212mg) in triethylamine (5.0mL), bis (tributyltin) (2mL) and tetrakis (triphenylphosphine) palladium(0) (47mg) were added. The mixture was heated at 100℃ in a sealed bottle for 48h. The solvent was removed under reduced pressure and the residue was purified successively by column chromatography (VHex/VetOAc= 2:1) on silica gel to give compound Ⅳ (274mg, 78.7%) as a red oil, TLC (VHex/VetOAc=2:1) $R_f$= 0.75. IR($cm^{-1}$): 2954, 2920, 2851, 1612, 1486; MS: 527 (M+1); $^1$HNMR: δ0.9~1.36 (m,27H), 3.00 (s,6H), 6.78 (d,2H), 7.12 (dd,1H), 7.58 (d,1H), 7.68 (s,1H), 7.82 (d,2H), 7.95 (s,1H).

**2.2.5** Radiolabeling of [$^{131}$I]IMPY

The tin compound Ⅳ (50μg in 50μL of ethanol ), [$^{131}$I]sodium iodide (～37MBq), and 1mol/L HCl (100μL) were placed in a sealed vial. To this mixture, 100μL of $H_2O_2$ (3% solution in water) was added via a syringe at room temperature. The iodination reaction was terminated after 10 min by an addition of saturated $NaHSO_3$ and the resulting solution was neutralized by adding a saturated $NaHCO_3$ solution. The labeling yield was 80% determined by TLC and radiochemical purity of $^{131}$I-IMPY is above 95% after extracted by ethyl acetate.

## 3 Biodistribution in normal mice

[$^{131}$I]IMPY (0.2mL, 370kBq) was injected through tail veins into mice(18—20g, divided into 5 groups, 3 for each). The mice were sacrificed at regular intervals (2, 30, 60, 120 and 360min) postinjection. The organs of interest (heart, muscle, lung, kidney, spleen, liver, skin, brain, blood etc.) were dissected and weighed, prepared for counting, and the uptake of each organ was expressed as percentage of injection dose per gram.

## 4 Results and discussion

The chemical structure of the labeling precursor 2-(4′-dimethylaminophenyl) - 6-(tributylstannyl) imidazo [1,2-α] pyridine (Ⅳ) and all its intermediates were verified by IR, HNMR and MS. We coupled 2-amino-5-bromopyridine (commercially available) and 2-bromo-4'-nitro- acetophenone (commercially available**)** to afford compound Ⅰ, which was converted to amino compound by reduction of the nitro group with tin (Ⅱ) chloride under acidic conditions to form compound Ⅱ. Compound Ⅱ was coupled with iodomethane to afford compound Ⅲ which is subsequently converted to the bromo derivative of target compound. The bromo to iodo transformation was achieved via a tributyltin intermediate, Ⅳ. The tributyltin intermediate Ⅳ was prepared by a palladium(0)-catalyzed coupling reaction with bis (tributyltin), with which the tributyltin group replaced the bromo group. The total yield of the multi-steps was 28% and it was higher than the literature (8.8%)[6]. Final compound Ⅳ was treated with radioactive [$^{131}$I] sodium iodide in oxidative condition ($H_2O_2$) to produce the labeled compound [$^{131}$I]IMPY in excellent yields. The labeling yield was 80% determined by TLC and radiochemical purity of $^{131}$I-IMPY is above 95% after extracted by ethyl acetate. The $R_f$ value of $^{131}$I- and $^{131}$I-IMPY is 0—0.1 and 0.9—1.0, respectively.

Biodistribution studies (Table 1) in normal mice showed that [$^{131}$I]IMPY exhibited excellent brain uptake (7.374±4.797)%ID/g at 2min) and fast washout (0.967±0.409)%ID/g at 60min), suggesting low non-specific binding in vivo, which is highly desirable for Aβ plaque imaging agents. The radioactivity in blood is relatively low at all time points measured, indicating the low imaging background will be achieved. The tracer seems to distribute in high blood flow areas, such as heart, liver and lung (Table 1) at early time points. At later points, the compounds were rapidly cleared from these organs.

**Table 1** Biodistribution of $^{131}$I-IMPY in mice (%ID/g, *n*=3)

| Organ | 2min | 30min | 60min | 120min | 360min |
|---|---|---|---|---|---|
| Brain | 7.37±4.79 | 1.45±0.50 | 0.97±0.409 | 0.52±0.10 | 0.21±0.05 |
| Muscle | 1.29±0.29 | 1.11±0.24 | 1.05±0.71 | 0.42±0.20 | 0.17±0.10 |
| Skin | 0.95±0.45 | 2.43±1.05 | 1.94±0.59 | 1.27±0.62 | 0.39±0.02 |
| Heart | 33.88±5.58 | 2.17±0.82 | 1.87±1.54 | 0.70±0.13 | 0.32±0.02 |
| Liver | 8.72±4.01 | 7.85±2.57 | 8.06±3.54 | 8.81±7.73 | 2.52±0.84 |
| Spleen | 0.72±0.25 | 2.76±1.02 | 1.63±0.87 | 1.82±0.24 | 0.82±0.28 |
| Lung | 68.75±45.33 | 6.00±2.29 | 5.71±5.01 | 2.61±0.72 | 1.37±0.39 |
| Kidney | 2.93±0.50 | 5.85±1.01 | 4.92±3.51 | 1.53±0.25 | 0.49±0.14 |
| Blood | 3.47±1.01 | 2.28±0.69 | 1.99±1.03 | 0.61±0.19 | 0.36±0.03 |

In summary, IMPY is a small molecular probe which has been developed and can readily enter the brain. Further evaluation of IMPY for in vitro binding properties and in vivo pharmacokinetic profiles is currently in process.

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