Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 5 September 2010, Revised 4 November 2010, Accepted 15 November 2010 Published online 17 February 2011 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1862

Radiosynthesis of tritium-labeled novel nitromethylene neonicotinoids compounds with NaB^3H_4

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Paichongding and Cycloxaprid are two novel neonicotinoids with good industrialization prospects for their high activity against imidacloprid-resistant pest. In this paper, the radiosynthesis of [$^3H_2$]-Paichongding and [$^3H_2$]-Cycloxaprid was achieved using a NaB^3H_4 reduction. The title compounds were obtained with chemical purities of 98.7 and 98.4%, and radiochemical purities of 97.3 and 98.6%, respectively. The labeled compounds could be used as radiotracers for further study of metabolism and toxicology.

Keywords: radiosynthesis; tritium-labeled; neonicotinoids; NaB^3H_4

Introduction

Dicyclic compound Paichongding and oxabridged compound Cycloxaprid (Figure 1) are two novel neonicotinoids with *cis*-configuration discovered by Qian *et al.* Both of them can effectively control sucking and biting insects of broad spectrum, such as cowpea aphids (*Aphis craccivora*), brown planthopper (*Nilaparvata lugens*), Fabricius (*Nephotettix bipunctatus*), and have low mammalian toxicity and favorable environmental profiles. Moreover, they exhibit significant activity against imidacloprid-resistant brown planthopper.[1,2]

It was found that Paichongding interacted at similar sites to imidacloprid and clearly discriminated between the high and low binding sites, potently interacting at the high-affinity imidacloprid binding site but only weakly at the low-affinity imidacloprid binding site.[3] The configuration of Paichongding is *cis*, while three proposals for modes of action (MOA) of neonicotinoids are based on *trans*-configuration.[4–6] The MOA and binding sites of *cis*-configuration nitromethylene neonicotinoids are still not clear. Isotope-substituted Paichongding and Cycloxaprid make it convenient to identify the MOA of these *cis*-configuration neonicotinoids. At the same time, the synthesis of labeled compounds is required in order to investigate their metabolism, degradation and environmental behavior in detail for future development. The use of radiolabeled compounds presents a promising approach for the detection and accurate analysis of compounds or products. Tritium, the general radionuclide, provides an opportunity for achieving the minimal disruption of the molecular structure, low raw material price and convenient synthesis.[7] Therefore, this paper describes the synthesis of tritium-labeled Paichongding ([$^3H_2$]-Paichongding, Scheme 1) and tritium-labeled Cycloxaprid ([$^3H_2$]-Cycloxaprid, Scheme 2), which could be used as radiotracers for the study of the MOA, metabolism, degradation and environmental behavior.

Experimental

General

Radiochemical sodium borohydride (80 mCi with a specific activity of 15 Ci/mmol) was purchased from American Radiolabeled Chemicals Inc. (USA). 6-Chloronicotinoyl chloride was obtained from Alfa Aesar. All other chemicals were obtained from commercial sources as chemically pure or higher grade and were used without further purification. Methanol for HPLC was of chromatographic grade from SK Chemicals (Korea) and ultrapure water (18.2 $M\Omega \cdot cm^{-1}$, 25°C) was prepared on Milli-Q academic instrument (Millipore, France). The other reagents were of analytical or higher grade and were used without purification. Melting points (mp) were recorded on Büchi B540 apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a BrukerAM-400 (400 MHz) spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. High-resolution mass spectra were recorded under electron impact (70 eV) condition using a MicroMass GCT CA 055 instrument. Analytical thin-layer chromatography (TLC) was carried out on precoated plates

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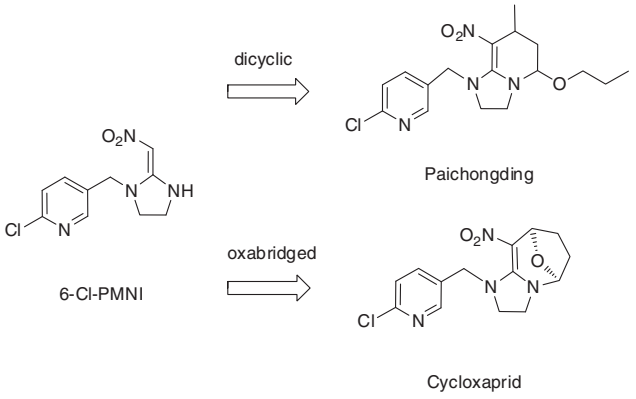
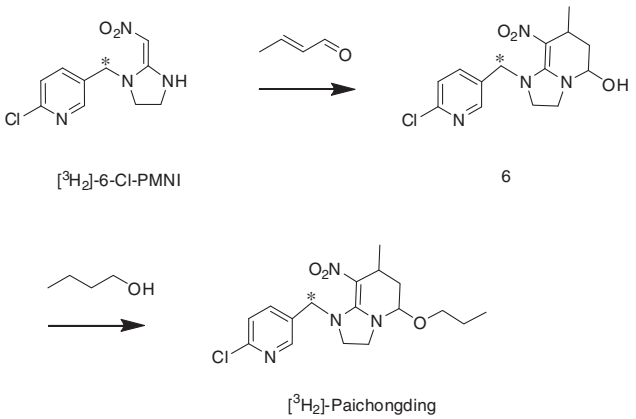
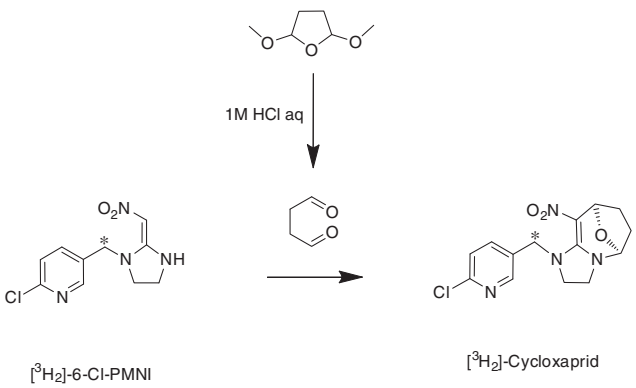


Figure 1. Structures of 6-Cl-PMNI, Paichongding and Cycloxaprid.



Scheme 1. Preparation of $[^3H_2]$-Paichongding with $[^3H_2]$-6-Cl-PMNI and crotonaldehyde (∗ the label site).



Scheme 2. Preparation of $[^3H_2]$-Cycloxaprid with $[^3H_2]$-6-Cl-PMNI and butanedial (∗ the label site).

(silica gel 60 F_{254}), and spots were visualized with ultraviolet light. X-ray diffraction was performed with a Bruker Smart 1000. TLC was performed with GF_{254} plates and radioactive TLC plates were scanned on Fujifilm BAS-1800II laser-based fluorescence and radioisotope imaging system (Fuji Co., Japan). Radioactivity was measured on WinSpectral-1414 liquid scintillation spectrometer (Wallac, Finland).

2-Chloro-5-hydroxymethylpyridine (2)

To the solution of freshly prepared NaB^3H_4 (52 mL, 80 mCi, 10 mCi/mmol, 8.0 mmol, NaOH/MeOH, 0.1 M) in anhydrous methanol (250 mL), 6-chloronicotinoyl chloride (**1**) (5 g, 28.6 mmol) was added in batches at room temperature (25°C), the reaction was kept for 4 h. After completion, the solution was concentrated in vacuum to 60 mL and treated with aqueous NaH_2PO_4 (1.0 M, 100 mL), followed by extraction with dichloromethane (5 × 100 mL). The organic layers were combined, dried over anhydrous Na_2SO_4 overnight. After the solvent was evaporated under reduced pressure, the residue was subjected to flash chromatograph (ethyl acetate/hexane 1:4, v/v) to afford white solid **2** (602 mg), yield 53%.

2-Chloro-5-chloromethylpyridine (3)

Thionyl chloride (15 mL) was added to the solution of **2** (602 mg) in $CHCl_3$ (30 mL), and the mixture was refluxing for 3 h and then cooled to room temperature. The solvent and thionyl chloride were removed under reduced pressure to give crude **3**, which was used in the next step without further purification.

N-(2-Chloro-5-pyridylmethyl)ethylenediamine (4)

To a solution of ethylenediamine (25 mL) in acetonitrile (5 mL) cooled in an salt ice bath (-15 to −10°C) was added **3** in acetonitrile (25 mL) dropwise by using a pressure equalizer funnel under argon within 45 min. The mixture was allowed to warm to room temperature and the reaction was continued overnight. To the resulting solution was added water (20 mL) followed by extraction with dichloromethane (6 × 40 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to afford crude **4**, which could be used for the next step without further purification.

2-Chloro-5-((2-(nitromethylene)imidazolidin-1-yl)$[^3H_2]$-methyl)pyridine ($[^3H_2]$-6-Cl-PMNI)

A mixture of *l*,*l*-bis(methylthio)-2-nitroethylene (1000 mg, 6.1 mmol) and *N*-(2-Chloro-5-pyridylmethyl)ethylenediamine in anhydrous ethanol (2 mL) was refluxed for 8 h affording a clear yellow solution. The solvent was removed *in vacuo* after cooling to room temperature. The crude product was purified by silica gel chromatography using dichloromethane:acetone (4:1, v/v) as an eluent to give white power with 820 mg, yield 40%.

1-((6-Chaloropyridin-3-yl)$[^3H_2]$-methyl)-7-methyl-8-nitro-1,2,3,5,6,7-hexahydroimidazo[1,2-a]pyridine-5-ol (6)

A mixture of $[^3H_2]$-6-Cl-PMNI (254 mg, 1 mmol), anhydrous acetonitrile (3 mL), crotonaldehyde (0.14 mL, 1.7 mmol) and acetic acid (0.09 mL) was stirred at 40–45°C for 26 h and the solvent was evaporated. The crude was purified by silica gel chromatography using dichloromethane: acetone (1:2, v/v) giving 131 mg of the pure material in 40% chemical yield, mp = 171.1–175.6°C; ^{1}HNMR (400 Mz, DMSO-d_6): δ 8.34 (d, J = 2.1 Hz, 1H), 7.80 (dd, J_1 = 2.4 Hz, J_2 = 8.3 Hz, 1H), 7.49 (d, J = 8.2 Hz, 1H), 6.29 (br d, J = 6.2 Hz, 1H), 4.82 (*m*, 1H), 4.73 (d, J = 15.5 Hz, 1H), 4.59 (d, J = 15.5 Hz, 1H), 3.58–3.74 (*m*, 4H), 3.11–3.16 (*m*, 1H), 1.88–1.95 (*m*, 1H), 1.69–1.75 (*m*, 1H), 1.03 (d, J = 6.6 Hz, 1H) ppm; ^{13}CNMR (400 Mz, DMSO-d_6):δ158.5, 149.6, 139.7, 132.6, 74.4, 109.3, 76.0, 51.9, 49.8, 45.2, 38.6, 28.3,

20.1 ppm; HRMS(EI^+) calcd for $C_{14}H_{17}N_4O_3^{35}Cl$ (M^+), 324.0989; found, 324.1014. calcd for $C_{14}H_{17}N_4O_3^{37}Cl$ (M^+), 3 26.0960; found, 326.0964; Anal. Calcd for $C_{14}H_{17}ClN_4O_3$: C, 51.78; H, 5.28; N, 17.25, Found: C, 51.91; H, 5.15; N, 16.95.[1]

1-((6-Chaloropyridin-3-yl)[$^3H_2$]-methyl)-5-propyloxy-7-methyl-8-nitro-5-propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-a]pyridine ([$^3H_2$]-Paichongding)

To the above compound **6** (131 mg, 0.4 mmol) in dichloromethane was added propan-1-ol (0.84 mL, 11.25 mmol) and hydrochloric acid (0.08 mL), the mixture was refluxed and stirred for 36 h, then the solvent was removed and the residue was purified by column chromatography (dichloromethane:acetone = 4:1, v/v) affording a pure yellow solid in 55% yield. mp = 130.2-131.9°C; ^{1}HNMR (400 Mz, $CDCl_3$):δ 8.31–8.33 (*m*, 1H), 7.86 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 0.5H), 7.82 (dd, J_1 = 2.4 Hz, J_2 = 8.2 Hz, 0.5H), 7.33 (dd, J_1 = 0.8 Hz, J_2 = 1.2 Hz, 0.5H), 7.31 (dd, J_1 = 0.8 Hz, J_2 = 1.2 Hz, 0.5H), 4.88 (d, J = 15.2 Hz, 0.5H), 4.82 (d, J = 15.2 Hz, 0.5H), 4.61–4.67 (*m*, 1H), 4.55 (dd, J_1 = 3.6 Hz, J_2 = 6.0 Hz, 0.5H), 4.50 (*t*, J = 3.6 Hz, 0.5H), 3.95–4.02 (*m*, 0.5H), 3.81–3.86 (*m*, 0.5H), 3.64–3.72 (*m*, 1H), 3.35–3.59 (5H), 2.14–2.21 (*m*, 0.5H), 1.99–2.01 (*m*, 1H), 1.75-1.82 (*m*, 0.5H), 1.54–1.65 (*m*, 2H), 1.22–1.27 (d, J = 6.8 Hz, 0.5H), 1.23 (d, J = 6.8 Hz, 0.5H), 0.95 (*t*, J = 6.2 Hz, 0.5H), 0.92 (*t*, J = 6.2 Hz, 0.5H) ppm; HRMS(EI^+) calcd for $C_{17}H_{23}N_4O_3^{35}Cl$ (M+)$^+$, 366.1459; found, 366.1487.[1]

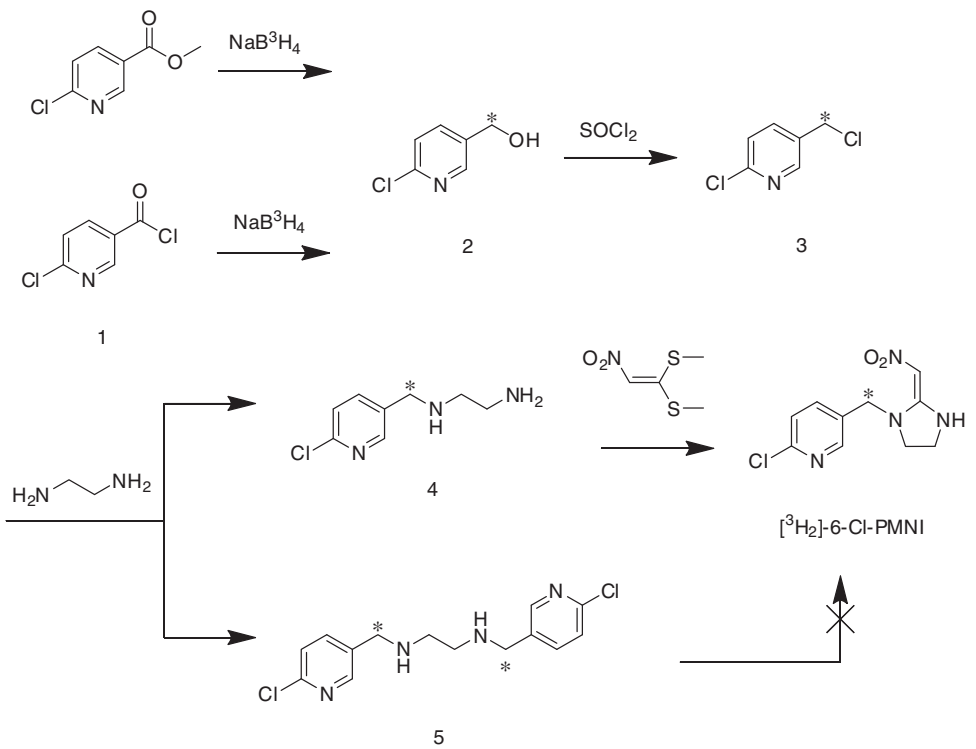
5-((6-Chloro-pyridin-3-yl)[$^3H_2$]-methyl)-7-nitro-11-oxa-2,5-diaza-tricyclo[6.2.1.0^{2,6}]undec-6-ene ([$^3H_2$]-Cycloxaprid)

To a mixture of tetrahydro-2,5-dimethoxy-furan (0.48 mL, 3.7 mmol) and [$^3H_2$]-6-Cl-PMNI (259 mg, 1.02 mmol) in acetonitrile (3 mL) was added hydrochloric acid (1.8 mL, 1 mol/L), the resulting solution was stirred at 35°C for 4 h and the reaction was monitored by TLC. Then the solution was neutralized with a saturated solution of $NaHCO_3$ and extracted with dichloromethane, the combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give an oily residue. Purification by silica gel chromatography using dichloromethane: acetone (4:1, v/v) as an eluent giving 165 mg of a canary yellow solid, yield 51%. mp = 149.0–150.0°C; ^{1}HNMR (400 Mz, DMSO-d_6):δ 8.35 (d, J = 2.4 Hz, 1H), 7.81 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 5.36–5.39 (s, 2H), 5.00 (d, J = 15.6 Hz, 1H), 4.68 (d, J = 15.6 Hz, 1H), 3.57–3.73 (*m*, 4H), 1.94–2.04 (*m*, 4H) ppm; ^{13}CNMR (400 Mz, DMSO-d_6):δ 155.6, 149.7, 149.6, 139.7, 132.6, 124.5, 109.6, 87.0, 75.1, 51.2, 50.3, 46.6, 31.9,31.7 ppm; HRMS (ESI^+) calcd for $C_{14}H_{16}N_4O_3^{35}Cl(M+H)^+$, 323.0911; found, 323.0912. calcd for $C_{14}H_{16}N_4O_3^{37}Cl$ $(M+H)^+$, 325.0811; found, 325.0895. calcd for $C_{14}H_{16}N_4O_3^{35}ClNa(M+Na)^+$, 345.0730; found, 345.0722. cacld for $C_{14}H_{16}N_4O_3^{37}ClNa(M+Na)^+$, 347.0701; found, 347.0692.[2]

The chemical and radiochemical purities of [$^3H_2$]-Paichongding and [$^3H_2$]-Cycloxaprid

The chemical purity was determined by HPLC. HPLC condition: Diamonsil C18 (2) 5 μm, 250 × 4.6 mm, solvent: acetonitrile/water = (65:35, v/v) for [$^3H_2$]-Paichongding and (75:25, v/v) for [$^3H_2$]-Cycloxaprid, flow: 1 mL/min. The retention time of [$^3H_2$]-Paichongding was 6.87 min, 12.16 min for its isomers, and [$^3H_2$]-Cycloxaprid was 6.44 min. The chemical purity of [$^3H_2$]-Paichongding and [$^3H_2$]-Cycloxaprid was 98.7 and 98.4%, respectively.

The radiochemical purity was measured with HPLC-LSC method, which is operated as follows: all the components were collected by flashing bottle after separation of labeled compounds by HPLC, the radioactivity of all the components(A_0)



Scheme 3. Preparation of [$^3H_2$]-6-Cl-PMNI with 6-chloronicotinoyl chloride (1) and NaB$^3H_4$ (* the label site).

and the target component(A_e) was measured with LSC. The ratio of A_e and A_0 indicates the radiochemical purity.[8] Radiochemical purity of [$^3H_2$]-Paichongding and [$^3H_2$]-Cycloxaprid was 97.3 and 98.6%, respectively.

Results and discussion

The radiosynthesis of tritium-labeled 2-chloro-5-((2-(nitromethylene) imidazolidin -1-yl)methyl)pyridine (6-Cl-PMNI) (Figure 1) has been reported by Latli *et al.*,[9] which involves high specific activity NaB^3H_4 and a multi-step microsynthesis. It required microreactor and difficult operation to achieve the final resolution. In this paper, a high-specific radioactivity methanolic NaB^3H_4 solution was first diluting by non-labeled $NaBH_4$, and this was taken to synthesize the target compounds offering the characteristics of a simple operation, high utilization ratio and providing sufficient quantities for further study. Various starting materials have been reported in introducing the tritium atoms into the flexible linkage.[9–11] The reduction of **1** by sodium borohydride in anhydrous methanol achieved higher radiochemical yield. As illustrated in the Scheme 3, compound **1** was reduced in methanol using NaB^3H_4 (80 mCi, specific activity 10 mCi/mmol) to give **2** after 3 h at room temperature. Refluxing with excess thionyl dichloride in chloroform, compound **2** was converted to **3** smoothly. When compound **3** was added dropwise into ethylenediamine in acetonitrile to obtain **4**, slow addition and low temperature were required to avoid the by-product **5** being easily formed. After the completion of the reaction, excess ethylenediamine was removed under reduced pressure to provide the crude compound **4**. Finally, the desired product [$^3H_2$]-6-Cl-PMNI was afforded by the condensation of **4** with l,l-bis(methylthio)-2-nitroethylene in ethanol under reflux.

The synthesis of Paichongding and Cycloxaprid has been reported in the earlier literature.[1,2] Reaction of half of the fresh synthesized [$^3H_2$]-6-Cl-PMNI with crotonaldehyde in acetonitrile afforded **6**, which was treated with propan-1-ol in acetonitrile, purified by silica gel column chromatography twice to prepare the product [$^3H_2$]-Paichongding. The overall chemical and radiochemical yield was 8.8 and 10.5%, respectively. The chemical purity was 98.7% and the radiochemical purity was 97.3%. The specific activity was 11.9 mCi/mmol.

The other half of [$^3H_2$]-6-Cl-PMNI reacted with butanedial to obtain [$^3H_2$]-Cycloxaprid, and the butanedial needs to be freshly prepared by 2,5-dimethoxytetrahydrofuran. The conversion of Cycloxaprid can also be achieved by the reaction of 6-Cl-PMNI and 2,5-dimethoxytetrahydrofuran in one pot. After the reaction was completed, the crude product was purified by silica gel column chromatography twice. The overall chemical and radiochemical yield was 20.7 and 29.7%, respectively. The chemical purity was 98.4%, while the radiochemical purity was 98.6%. The specific activity was 14.4 mCi/mmol.

Acknowledgements

This work was financial supported by National Basic Research Program of China (973 Program, 2010CB126104), Ministry of Agriculture of China (200803034), National High Technology Research and Development Program of China (863 Program 2010AA10A204) and National Science Foundation China Program Grant (20872034). This work was also partly supported by Science Foundation of Chinese University, Shanghai Foundation of Science and Technology (09391911800, 09XD1401300) and Shanghai Leading Academic Discipline Project, Project Number: B507. 111 Project (No. B07023).

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