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Research Article

Synthesis of stable isotope labeled PNU-95666

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Summary

Studies towards the synthesis of stable isotope labeled PNU-95666, a CNS agent currently under development for the treatment of Parkinson's disease, is described. The synthesis of non-labeled PNU-95666 starts using a large excess of methylamine. While the non-labeled methylamine is inexpensive and in plentiful supply, the stable isotope labeled form is expensive and limited availability. Different synthetic routes and conditions, including the use of different aminating agents and various amounts of methylamine were explored and new synthetic conditions were established. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: stable isotope labeled PNU-95666; sumanirole; CNS agent; [^{13}CD3] methylamine

Introduction

PNU-95666 is a highly selective D_2 agonist currently under development for the treatment of Parkinson's Disease.[1] The stable isotope labeled form is needed as an internal standard for LC/MS study, which requires it to be at least three mass unit higher than the corresponding parent compound.

Many synthetic routes for the synthesis of PNU-95666 have been reported[1–6] and in the most recent method the desired methylamino group was introduced by reaction of the chiral naproxen ester **1** with methylamine (Scheme 1).[6]

This route looks attractive for the synthesis of stable isotope labeled PNU-95666 since it allows us to use the commercially available $^{13}CD_3$ methylamine as the starting material. The drawback, however, is the use of a large excess of $^{13}CD_3$ methylamine (22 equivalents),[6] which is not only expensive but also of limited availability. One possible solution is to use ammonia or benzylamine instead of methylamine to form amine **4**. The reaction of amine **4** with much cheaper and widely available $^{13}CD_3$ methyl iodide should afford the desired

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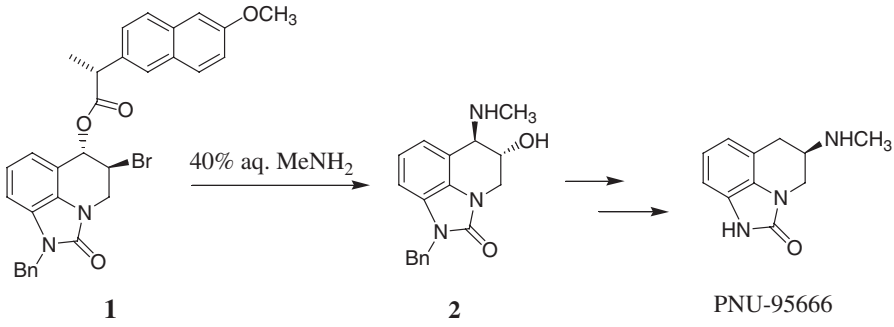
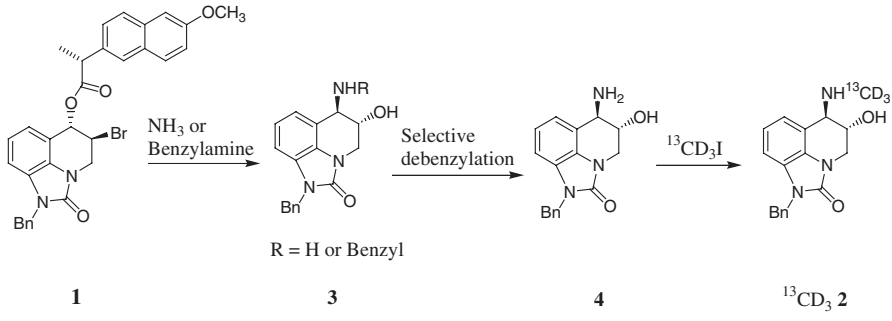


Scheme 1. Synthetic route for non-labeled PNU-95666



Scheme 2. Proposed synthesis of stable isotope labeled PNU-95666

intermediate **2** (Scheme 2). The other solution would be to lower the amount of $^{13}CD_3$ methylamine used to convert **1** to **2**.

Results and discussion

The reaction of naproxen ester **1** with ammonia was carried out with both concentrated aqueous ammonia (22 equivalents) and with ammonia in methanol (7 N, 22 equivalents). Only starting material was observed in both cases after overnight reaction at room temperature. No product was formed even under prolonged reflux. Apparently, ammonia itself is not nucleophilic enough for the reaction to occur. While the reaction of benzylamine with naproxen ester **1** looked attractive, we later learned that the selective debenzylation of **3** to **4** had been tried unsuccessfully.[7]

As the plan to use either ammonia or bezylamine to replace methylamine failed, we turned our attention to the use of less methylamine. Non-labeled amine **2** was made by the reaction of 22 equivalents of aqueous methylamine with naproxen ester **1** in acetonitrile at room temperature overnight in 83% yield. At least three equivalents of methylamine is needed for this transformation: one for amidation, one to open the epoxide, and one to neutralize the liberated HBr (Scheme 3).

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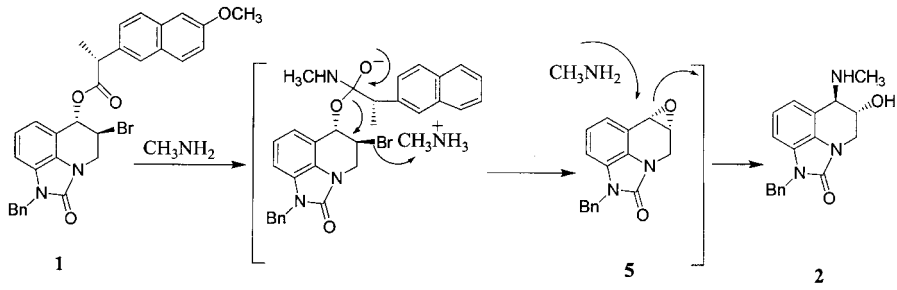

Scheme 3. Reaction mechanism

To find out how much of the reagent is practically needed for the reaction to proceed, we started with three different experiments: stirring a solution of naproxen ester **1** with: (1) 22 equivalents, (2) 10 equivalents, and (3) 6 equivalents of 40% methylamine in acetonitrile at room temperature. The reaction started as a suspension (naproxen ester **1** is sparely soluble in the reaction media) and ended up as a homogeneous solution, as the reaction proceed to completion. All three reaction went to completion: while reaction (1) and reaction (2) became homogeneous solutions within a reasonable 18 h and a manageable 96 h respectively, reaction (3) took a prohibitive 216 h.

This work and the fact that there was no other good starting material available (either labeled or non-labeled) led us to carry out the synthesis as outlined in Scheme 4.

Commercially available $^{13}CD_3NH_2$ hydrochloride was first converted to the aqueous free amine, which was then reacted with naproxen ester **1** in acetonitrile to afford stable isotope labeled amino alcohol **2** in 89% yield after flash chromatography purification. Amino alcohol **2** was treated with 1 equiv of BuLi followed by benzenesulfonyl chloride, which selectively sulfonates the hydroxyl, and upon base treatment, causes ring closure to the aziridine **6** in 55% yield. Reductive ring opening and debenzylation was achieved in one step when aziridine **6** was treated with lithium in ammonia, and the final compound was isolated as the maleic acid salt in 70% yield. The chemical purity of $^{13}CD_3$ PNU-95666 was $>97\%$ when analyzed by HPLC.† The NMR spectrum of $^{13}CD_3$ PNU-95666 showed no *N*-methyl CH_3 signal and no non-labeled molecular ion was observed when analyzed by mass spectrometry.

Experimental

$^{13}CD_3$ Methylamine hydrochloride (99% C-13 and 99.5 deuterium enrichment) was purchased from Aldrich. ^{1}H NMR spectra were recorded on a

†HPLC condition: column, Zorbax RX-C8 (5 μ, 4.6 × 250 mm); mobile phase, methanol/water/triethylamine (500/500/1); flow-rate, 1 ml/min; UV detector, 282 nm.

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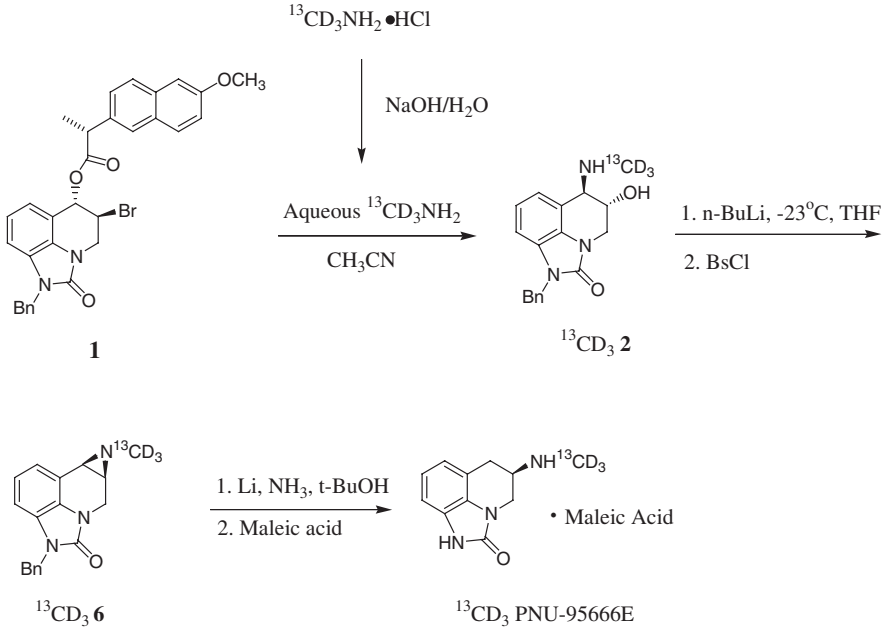

Scheme 4. Synthetic of stable isotope labeled PNU-95666

Bruker 300 MHz spectrometer. $CDCl_3$ was used as the NMR solvent unless otherwise indicated. ^1H NMR spectra are reported relative to TMS. Mass spectra were obtained with a Finnigan TSQ-700 mass spectrometer. HPLC analysis was performed on a Perkin Ekmer ISS 200 HPLC instrument. All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated.

$^{13}CD_3$ (5R,6R)-1-Benzyl-5-hydroxy-6-(methylamino)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one ***2***

A solution of 10 g of sodium hydroxide (250 mmol) in 10 ml of water was added dropwise with stirring to a solution of 14 g of $^{13}CD_3NH_2$ hydrochloride (197 mmol) in 10 ml of water at room temperature. After the reaction was complete, free $^{13}CD_3NH_2$, along with water, was distilled under vacuum into a solution of 10 g of naproxen ester **1** (17.6 mmol) in 150 ml of acetonitrile. The resulting reaction mixture was stirred at room temperature for 72 h. TLC analysis (silica gel plate, hexane/EtOAc/MeOH 10/10/1) found no remaining starting material. Excess $^{13}CD_3NH_2$ and solvent was removed under reduced pressure. The residue was suspended in 115 ml of EtOAc, treated dropwise with 37.5 ml of 5% HCl, and stirred at room temperature for 10 min. The two layers were separated and the EtOAc layer was washed with water (2×37.5 ml). The combined aqueous phase was washed with EtOAc

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(2×37.5 ml), and then, basified with dropwise addition of 19 ml of 10 M NaOH at 0°C. Free amino alcohol **2** was extracted with EtOAc (2×75 ml), washed with water (2×35 ml), and dried over anhydrous sodium sulfate. Filtration and solvent removal left an oily residue. The residue was then purified by flash chromatography (silica gel column, eluted with hexane/EtOAc/MeOH 3/3/1) to afford 4.88 g (89%) of $^{13}CD_3$ labeled amino alcohol **2**, which was found to be identical with an authentic sample when analyzed by TLC (silica gel plate, hexane/EtOAc/MeOH 1/1/1, Rf 0.41). Mass spectrum (ESI): m/z 314 (MH^+). ^{1}H NMR (DMSO-d6, 300 MHz), δ: 7.35 (m, 5H), 7.0 (m, 3H), 5.0–5.15 (m, 3H), 4.25, (m, 1H), 3.78 (m, 2H), 3.50 (m, 1H).

$^{13}CD_3$ (7aS,8aR)-4-Benzyl-8-methyl-7,7a,8,8a-tetrahydroazireno [2,3-c] imidazo [4,5,1-ij] quinolin-5(4H)-one **6**

n-Butyl lithium in hexane (2.5 M, 6.6 ml, 16.5 mmol) was added dropwise to a solution of 4.88 g of $^{13}CD_3$ labeled amino alcohol **2** (15.1 mmol) in 25 ml of THF kept at −25°C. The resulting reaction mixture was stirred at −25°C for 15 min and then 2.116 ml of benzensulfonyl chloride (16.5 mmol) was added slowly, keeping the temperature below −23°C. The solution was stirred at −25°C for 1 h, warmed up to room temperature over 60 min, and stirred for 10 min. Saturated aqueous $NaHCO_3$ solution (11 ml) was added, followed by 75 ml of water. The solid precipitated was collected by filtration and washed with water (2×5 ml) to afford 2.50 g (55%) of $^{13}CD_3$ labeled aziridine **6** as a colorless solid after drying. Aziridine **6** was used directly in next reaction without further purification and identification.

$^{13}CD_3$ PNU-95666E

$^{13}CD_3$ Aziridine **6** (500 mg, 1.7 mmol) was charged into a 3-necked flask, which was then attached to a dewar condenser. The condenser and flask were cooled in dry ice/acetone bath, and NH_3 gas was let in to the flask until about 10 ml of liquid ammonia had been condensed. *t*-Butyl alcohol (0.5 ml) was added to the solution, followed by 97 mg of lithium (13.8 mmol) in small pieces. The reaction mixture was refluxed for 90 min at −33°C and then quenched by the slow addition of 1 ml of water. After quench, the slurry was stirred for 15 min at reflux and warmed up to room temperature to remove ammonia. More water (4.5 ml) and hexane (10 ml) was added and the resulting mixture was stirred until all the solid dissolved. The phases were allowed to separate and the aqueous phase was washed with more hexane (10 ml). Most of the water was removed by concentration and the desired product was extracted by stirring the aqueous residue with EtOAc overnight (2×150 ml). Ethyl acetate extracts were combined and the solvent was removed to afford a gummy residue. The residue was dissolved in 3 ml of ethanol. The ethanol solution was brought to gentle reflux and a warm solution of 200 mg of maleic acid in 3 ml

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of ethanol was added slowly over 30 min. Crystals started to appear while the addition was proceeding. The resulting suspension was cooled to 0°C and filtered. The solid collected was again taken up in 7 ml of ethanol. The ethanol suspension was heated to reflux, cooled to room temperature, and kept in a refrigerator overnight. The solid was collected by filtration and dried under vacuum to afford 382 mg (70%) of $^{13}CD_3$ PNU-95666 E. The chemical purity was 97.2% when analyzed by HPLC.[†] Mass spectrum (ESI): m/z 208 (MH^+). No non-labeled PNU-95666 (m/z 204) was observed. NMR (DMSO-d6, 300 MHz): δ 3.04 (dd, 1H, J = 16.5, 6), 3.23 (dd, 2H), 3.9–4.06 (m, 3H), 6.02 (s, 2H, maleic acid CH = CH), 6.85–6.97 (m, 3H), 8.55 (br s, 2H), 10.83 (s, 1H).

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