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Synthesis of (E)-N-[Methyl-d_3]- 4-(3-pyridinyl)-3-buten-1-amine, a Deuterated Analogue of the Nicotinic Agonist RJR-2403

Peter A. Crooks*[1], Alain Ravard[1], and Gary D. Byrd[2]

[1]College of Pharmacy, Division of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40536-0082, USA

[2]Research and Development, R. J. Reynolds Tobacco Company, Winston-Salem, NC 27102, USA

SUMMARY

The synthesis of (E)-N-[methyl-d_3]-4-(3-pyridinyl)-3-buten-1-amine ([methyl-d_3]RJR 2403; [methyl-d_3]metanicotine) is reported. The incorporation of deuterium was performed during the first step of the synthesis via N-methylation of the pyrrolidine nitrogen of racemic nornicotine with [methyl-d_3]iodomethane, in the presence of n-BuLi at -70°C to afford racemic [methyl-d_3]nicotine in high yield (91%). The pyrrolidine ring was then cleaved with ethyl chloroformate to give (E)-N-[methyl-d_3]-N-ethyloxycarbonyl-4-(3-pyridinyl)-3-buten-1-amine; in this reaction, elimination of HCl occurred during heating of the intermediate N-[methyl-d_3]-N-ethyloxycarbonyl-4-chloro-4-(3-pyridinyl)butan-1-amine under vacuum (0.5 mm Hg). The last step of the synthesis, i.e. the removal of the N-carbamoyl group, was achieved via acidic hydrolysis with concentrated aqueous hydrochloric acid, to afford [methyl-d_3]metanicotine in 82% overall yield. The isotopic purity of the sample was determined by mass spectrometry and calculated to be 97.6 atom % deuterium.

Key words: Deuterated RJR-2403, nicotine metabolite, tobacco alkaloid, [methyl-d_3]metanicotine.

INTRODUCTION

Metanicotine (N-methyl-4-(3-pyridinyl)-3-buten-1-amine) is a major alkaloid found in the leaves of *Duboisia Hopwoodii*[1] (up to 20% is present in some species). It has also been reported that metanicotine is found in some *Nicotiana* plants[2], and is a constituent of tobacco smoke[3].

* Correspondence

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Because of the inverse association between tobacco smoking and neurodegenerative disease[4-6], compounds which are structurally related to nicotine may be of value as therapeutic agents for the treatment of certain central nervous system disorders. Articles describing analogues of nicotine [7,8] as well as metanicotine and analogues of metanicotine[9], as potential drugs for the treatment of senile dementia of the Alzheimer's type, have recently been published, and metanicotine (RJR 2403) is currently being investigated as a nicotinic agonist with CNS selectivity.[10] Metanicotine may have clinical potential due to its high selectivity for CNS nicotinic acetylcholine receptor subtypes relative to peripheral ganglionic and muscle-type nicotinic acetylcholine receptors. Thus, the availability of a deuterium-labeled form of metanicotine will provide an important tool for metabolic and pharmacokinetic studies of this interesting compound.

We have previously reported the synthesis of several deuterium-labeled metabolites of nicotine[11-13] which have successfully been used as standards in the development of direct methods for the analysis of biological samples using liquid chromatography/mass spectrometry. In this present communication we report on a synthesis of [methyl-d_3]metanicotine which is based on a modification of a previous method of Acheson et al.[14].

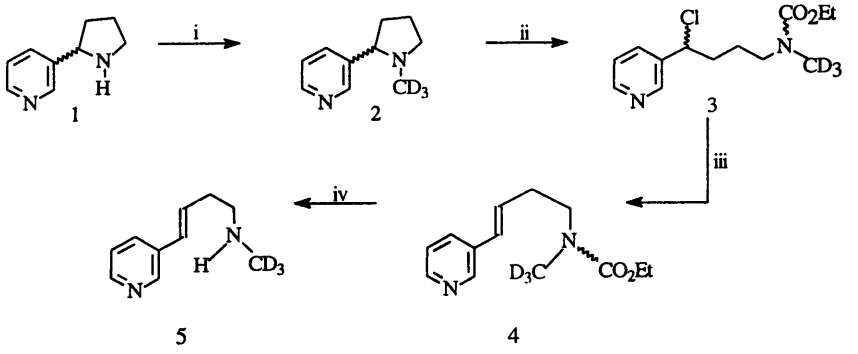
RESULTS AND DISCUSSION

The preparation of [methyl-d_3]metanicotine was carried out via the synthetic route illustrated in the Scheme. The precursor utilized was *rac*-nornicotine (**1**), which was prepared according to a previously reported procedure[15,16]. The first step in the synthesis was the preparation of the key intermediate, *rac*-[methyl-d_3]nicotine. N-Methylation of nicotinic compounds with iodomethane has previously been reported in the literature[17]; however, even when only one equivalent of iodomethane is utilized in this reaction, two isomeric N-methyl compounds are formed as a result of indiscriminate methylation of either of the nitrogen atoms of the nicotine molecule. Methylation of nornicotine with iodomethane/acetic acid affords a complex mixture containing nicotine, N-1-methylnornicotinium iodide, N-1-methylnicotinium iodide, and N-1'-methylnicotinium iodide[18]. Thus, we decided to use an alternative approach involving initial formation of the lithium salt of nornicotine, by addition of n-butyl lithium at -70°C, followed by N-methylation with commercially available [methyl-d_3]iodomethane. The desired *rac*-[methyl-d_3]nicotine (**2**) was obtained in high yield (91%). The isotopic purity of this key intermediate was determined by high resolution mass spectrometry to be 99.5 atom %D.

Ring opening of the pyrrolidine ring of rac-[methyl-d_3]nicotine was carried out utilizing a modification of a previously reported procedure.[14] A solution of *rac*-[methyl-d_3]nicotine (**2**) in

anhydrous methylene chloride was added to a solution of ethyl chloroformate in the same solvent. After refluxing the mixture for 4 hours, N-[methyl-d_3]-N-ethyloxycarbonyl-4-chloro-4-(3-pyridinyl)butan-1-amine (**3**) was obtained in 75% yield. The ^{13}C NMR spectrum of (**3**), after purification by column chromatography, clearly indicated the presence of two isomeric species which were not separable by TLC, and were attributable to the syn-and anti-isomers of **3**. Two carbon resonances were observed for the carbonyl carbon at δ 156.3 and 156.2 ppm, and for the C-6 and C-3 carbons of the pyridine ring, at δ 149.3 and 149.2 ppm, and δ 137.2 and 137.1 ppm, respectively; the C-Cl resonance exhibited singlets at δ 25.0 and 24.6 ppm.

The dehydrochlorination step to afford **4** was carried out by heating the chloro compound **3** under vacuum (0.5 mm Hg) until evolution of HCl gas had ceased. From this reaction compound **4** was obtained in 77% yield after column chromatography of the crude product. The ^{13}C-NMR spectrum of **4** also indicated it to be a mixture of geometrical isomers. The hydrolysis of **4** to [methyl-d_3]metanicotine (**5**) was achieved by refluxing with concentrated aqueous hydrochloric acid (10 N) for 20 hours to afford the desired product in 84% yield. The ^1H NMR spectrum of **5** indicated the presence of only the (E)-form of [methyl-d_3]metanicotine. In the deuterium-coupled ^{13}C-NMR spectrum of **5**, the N-CD_3 group was observed as a 1:3:6:7:6:3:1 septet at δ28.95 ppm, with J_{CD} =21.9 Hz. The free base of **5** was converted to its fumarate salt, and the isotopic purity was determined using electrospray mass



i) n-BuLi, CD_3I, THF, -70°C 15min; ii) $ClCO_2Et$, CH_2Cl_2 reflux

iii) heating under vacuum (0.5 mm Hg); iv) 10N HCl, reflux

Scheme: Synthesis of [methyl-d_3]metanicotine

spectrometry. The base peak in the mass spectrum of the labeled compound was the $(M+H)^+$ ion at *m/z* 166. The intensities of the ions corresponding to 1, 2, and 3 Daltons less than the $(M+H)^+$ peak were recorded and the relative ratios to the base peak calculated. These ratios were corrected for the corresponding ions at 1, 2, and 3 Daltons less than $(M+H)^+$ in the unlabeled analog when analyzed under the same conditions. The d_3-labeled material was found to be 97.6 atom % by this method. The small loss (~2%) of deuterium observed in the conversion of [methyl-d_3]nicotine to [methyl-d_3]metanicotine most likely results from deuterium exchange during the N-decarbamoylation of (**4**) (step iv, Scheme), which is carried out under extremely acidic conditions.

EXPERIMENTAL

[Methyl-d_3]iodomethane 99.5% gram atom deuterium was obtained from Cambridge Isotope Laboratories (Andover, MA), all other chemicals and solvents were purchased from the Aldrich Chemical Company (Milwaukee, WI). Silica gel plates (2.5 x 7.5 cm), fluorescent at 254 nm, were purchased from Diamond Whatman International Ltd. (Hillboro, OR). Column chromatographic separations were carried out using silica gel, 200-400 mesh, 60Å, from the Aldrich Chemical Company (Milwaukee, WI). Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. ^1H, ^{13}C and two-dimensional HETCOR and COSY NMR spectra were obtained on a Varian VXR-300 MHz spectrometer (Palo Alto, CA); spectra were run at 21°C in $CDCl_3$ or D_2O using tetramethyl silane (TMS) or the sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP) as internal standard. Electrospray mass spectra were recorded on a Micromass Quattro II mass spectrometer with an electrospray interface (Manchester, UK). A methanol solution of the labeled compound was introduced into the instrument in a flow of methanol at 0.1 mL/min. The source was maintained at 80°C with a cone voltage of 5 V. The mass spectrometer was scanned repetitively from *m/z* 10 to 250 at a rate of 2 s per scan. Electron impact (EIMS; low and high resolution) and fast atom bombardment mass spectra (FAB-MS) were recorded using a Kratos Concept 1-H mass spectrometer (Manchester, UK).

(±)-N-[Methyl-d_3]nicotine (**2**):

A solution of n-BuLi in n-hexane (1.8 mL, 42.5 mmol) was added to a solution of (±)-nornicotine (**1**)[17,18] (6g, 40 mmol) in anhydrous THF (75 mL at -70°C, dry-ice/acetone bath). The solution was stirred for 15 min, then [methyl-d_3]iodomethane (7.2g, 50 mmol) was added. The mixture

was stirred for an additional 30 min and then allowed to warm to 0°C. Water (25 mL) was added to the mixture, followed by diethyl ether (25 mL). The solution was decanted and the aqueous layer extracted with ether (3x25 mL). The combined organic extracts were dried over K_2CO_3, filtered and concentrated. The resulting crude product was then distilled (52°-55°C/0.1 mm Hg) to afford purified (±)-N-[methyl-d_3]nicotine (**2**) (6g, 91%).

^{1}H NMR ($CDCl_3$): δ 8.82 (s, 1H, H-2), 8.79-8.76 (m, 1H, H-6), 8.00-7.96 (m, 1H, H-5), 7.56-7.53 (m, 1H, H-4), 3.55-3.50 (m, 1H, H-2'), 3.39-3.34 (m, 1H, H-5'a), 2.60-2.47 (m, 2H, H-3'a+H-5'b), 2.34-2.18 (m, 1H, H-3'b), 2.18-1.94 (m, 2H, H-4') ppm. ^{13}C NMR ($CDCl_3$): δ 149.5 (C-2), 148.6 (C-6). 138.7 (C-3), 134.7 (C-4), 123.5 (C-5), 68.7 (C-2'), 56.9 (C-5'), 35.1 (C-3'), 22.6 (C-4') ppm. EI-MS: *m/z* 165 (11.5, $M^{+\cdot}$), 136 (24.9), 87 (100).

N-[Methyl-d_3]-N-ethyloxycarbonyl-4-chloro-4-(3-pyridinyl)butan-1-amine (**3**):

A solution of (±)-[methyl-d_3]nicotine (**2**) (5.8g, 35.1 mmol) in anhydrous CH_2Cl_2 (30 mL) was added dropwise to a solution of ethyl chloroformate (4.20g, 38.6 mmol) in anhydrous CH_2Cl_2 (20 mL), under nitrogen and at ambient temperature. The resulting solution was refluxed for 4 hours, then allowed to cool to ambient temperature before removing the solvent by evaporation under vacuum. The resulting crude material was purified by column chromatography over silica gel using methanol in chloroform (4:96, v/v) as eluent and afforded purified N-[methyl-d_3]-N-ethyloxycarbonyl-4-chloro-4-(3-pyridinyl)butan-1-amine (**3**) (7.2g, 75%).

^{1}H NMR ($CDCl_3$): δ 8.62 (s, 1H, H-2), 8.57 (d, 1H, J=5 Hz, H-6), 7.76 (m, 1H, H-5), 7.32 (dd, 1H, J=8,5 Hz, H-4), 5.04-4.86 (m, 1H, CHCl), 4.12 (q, 2H, J=7 Hz, OCH_2), 3.46-3.24 (m, 2H, CH_2N), 2.18-1.93 (m, 2H, CH_2CHCl), 1.84-1.52 (m, 2H, CH_2CH_2N), 1.24 (t, 3H, J=7 Hz, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ 156.3 and 156.2 (C=O two isomers), 149.3 and 149.2 (C-6 two isomers), 148.0 (C-2), 137.2 and 137.1 (C-3 two isomers), 134.2 (C-4), 123.4 (C-5), 60.9 (OCH_2), 59.9 (CHCl), 47.2 (CH_2N), 36.5 (CH_2-CH_2N), 25.0 and 24.6 (CH_2-CHCl, two isomers), 14.5 (CH_3) ppm.

(E)-N-[Methyl-d_3]-N-ethyloxycarbonyl- 4-(3-pyridinyl)-3-buten-1-amine (**4**):

N-[methyl-d_3]-N-ethyloxycarbonyl-4-chloro-4-(3-pyridinyl)butan-1-amine (**3**) (7g, 25.6 mmol) was heated under vacuum (0.5 mm Hg) until no more evolution of HCl gas was observed. The resulting dark oily residue was then purified by column chromatography over silica gel using methanol in chloroform (4:96, v/v) as eluent and afforded 4.7g (77%) of pure compound (**4**).

^{1}H NMR ($CDCl_3$): δ 8.55 (s, 1H, H-2), 8.43 (dd, 1H, J=5,1.5 Hz, H-6), 7.64 (m, 1H, H-4), 7.20 (dd, 1H, J=8,5 Hz, H-5), 6.44-6.21 (m, 2H, C<u>H</u>=C<u>H</u>-CH_2), 4.11 (q, 2H, J=7Hz, OCH_2), 3.40 (m, 2H, CH_2N), 2.46 (m, 2H, CH_2), 1.22 (t, 3H, J=7 Hz, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ 155.8 (C=O), 147.6 (C-6), 147.3 (C-2), 132.3 (C-3), 131.8 (C-4), 129.1 and 128.9 (=<u>C</u>H-CH_2, two isomers), 127.7 (Pyr-<u>C</u>H=), 122.7 (C-5), 60.5 (OCH_2), 47.7 and 47.6 (CH_2N, two isomers), 31.3 and 31.0 (CH_2, two isomers), 14.1 (CH_3) ppm.

<u>(E)-N-[Methyl-d_3]- 4-(3-pyridinyl)-3-buten-1-amine (**5**):</u>

A solution of (E)-N-[methyl-d_3]-N-ethyloxycarbonyl-4-(3-pyridinyl)-3-buten-1-amine (**4**) (4.5g, 18.96 mmol) in 10N aqueous HCl (25 mL) was refluxed for 20 hours. The resulting solution was then cooled to ambient temperature, basified with a 20% ^{w}/v aqueous solution of NaOH, and extracted with chloroform (3x30 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated to give a crude oil, which was purified by vacuum distillation (99°-101°C/0.4 mm Hg) to afford 2.6g (84%) of compound **5**.

^{1}H NMR ($CDCl_3$): δ 8.56 (d, 1H, J=2 Hz, H-2), 8.43 (dd, 1H, J=5,2 Hz, H-6), 7.65 (m, 1H, H-4), 7.21 (dd, 1H, J=8,5 Hz, H-5), 6.46-6.24 (m, 2H, pyr-C<u>H</u>=C<u>H</u>-CH_2), 2.73 (t, 2H, J=7 Hz, CH_2N), 2.41 (q, 2H, J=7 Hz, CH_2), 1.19 (broad s, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ 148.0 (C-6), 147.8 (C-2), 132.8 (C-3), 132.3 (=<u>C</u>H-CH_2), 130.7 (C-4), 127.8 (C-5), 123.2 (pyr-<u>C</u>H=), 50.9 (CH_2N), 33.5 (CH_2) ppm.

<u>(E)-N-[Methyl-d_3]- 4-(3-pyridinyl)-3-buten-1-amine monofumarate:</u>

Fumaric Acid (1.7g, 14.5 mmol) was added to a solution of (E)-N-[methyl-d_3]-4-(3-pyridinyl)-3-buten-1-amine (**5**) (2.4g, 14.5 mmol) in absolute EtOH (15 mL) and the resulting mixture was heated until complete dissolution occurred. A gummy residue was obtained after removal of solvent by rotary evaporation, which slowly solidified on drying to yield 4g (99%) of the monofumarate salt of (**5**), mp =116-117°C.

^{1}H NMR (D_2O+TSP): δ 8.66 (s, 1H, H-2), 8.54 (d, 1H, J=6Hz, H-6), 8.38 (m, 1H, H-4), 7.82 (dd, 1H, J=8, 6Hz, H-5), 6.71-6.67 (m, 1H, Pyr-C<u>H</u>=), 6.55-6.40 (m, 3H, =C<u>H</u>-CH_2 superimposed with s, CH=CH fumarate), 3.25 (t, 2H, J=7Hz, CH_2N), 2.70 (q, 2H, J=7Hz, CH_2) ppm. ^{13}C NMR (D_2O+TSP): δ 176.2 (C=O, fumarate), 144.0 (C-6), 143.5 (C-2), 142.8 (C-4), 138.1 (C-3), 137.7 (CH=CH fumarate), 133.5 (CH=<u>C</u>H_2), 129.9 (pyr-<u>C</u>H=), 128.7 (C-5), 50.2 (CH_2N), 31.7 (CH_2) ppm. FAB-MS (positive mode): *m/z* 166 $((M+H)-C_4H_4O_4)^+$.

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