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Research Article

Synthesis of ^{15}N-labelled nornicotine and ^{15}N-labelled nicotine

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Summary

The synthesis of ^{15}N-labelled nornicotine **2** and ^{15}N-labelled nicotine **1** is described via the reductive aminocyclization of a 1,4-ketoaldehyde with a corresponding amine in the presence of sodium cyanoborohydride. Yields of 30% and 26%, respectively, were obtained from 3-bromopyridine. The ^{15}N-label has been easily introduced from ammonium-^{15}N-labelled chloride as an inexpensive label source. As far as we are aware, this is the first synthesis of ^{15}N-labelled nicotine. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: reductive aminocyclization; methylation; ^{15}N-labelled compounds; nicotine

Introduction

The *N*-demethylation of nicotine [3-(1-methyl-pyrrolidin-2-yl)-pyridine] to nornicotine (3-pyrrolidin-2-yl-pyridine) is an important reaction both

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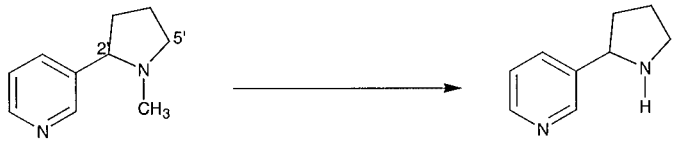


1 nicotine **2** nornicotine

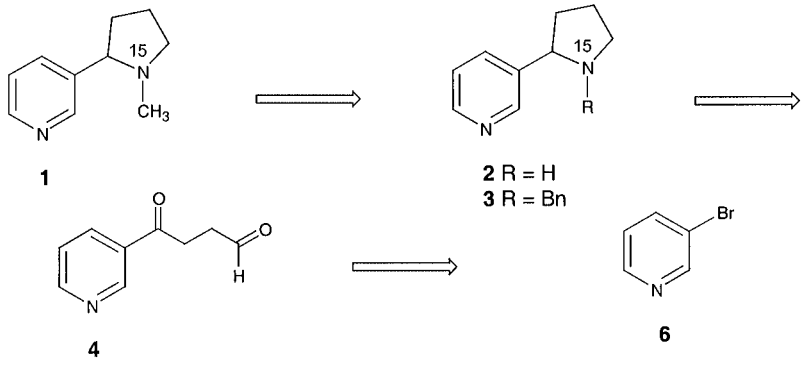
Scheme 1.

in terms of the detoxification of this poison by human hepatic tissue[1,2] and in the quality of smoking tobacco.[3] While this reaction has been recognized for more than 50 years in *Nicotiana* species,[4] the exact mechanism of the process in plants is still unclear (Scheme 1). In animal cells, a P450-dependent oxidation occurs, in which the C5′ pro-(*S*) hydrogen is lost.[5] A number of mechanisms for the demethylation of nicotine in plants have been proposed, including oxidation at the C-2′ position, oxidation at the C-5′ position or oxidation at the *N*-methyl group.[6,7] Evidence has been presented eliminating the two former possibilities and showing that the intermediary of nicotine-1-*N*-oxide is improbable[8]. Recently, a mechanism involving oxidation at the *N*-methyl group has received strong support based on *in vivo* NMR using nicotine labelled with either ^{2}H or ^{13}C in the methyl group.[9]

In order to examine in further detail the demethylation of nicotine and the further metabolism of nornicotine, specifically [^{15}N-*pyrrolidinyl*]-labelled nicotine is desirable. While a number of previous investigations report the use of ^{15}N-nicotine, this has exclusively been produced by biosynthesis from $K^{15}NO_3$ in nutrient solutions. Tobacco plants (*Nicotiana rustica*, *Nicotiana glauca* and *Nicotiana glutinosa*) were grown in these solutions to produce chiral ^{15}N-labelled nornicotine and ^{15}N-labelled nicotine.[10–12] To our knowledge, no synthesis of specifically [^{15}N-*pyrrolidinyl*]-labelled nicotine **1** or nornicotine **2** has previously been reported in the literature.

Results and discussion

In this paper, we describe the synthesis of ^{15}N-labelled nicotine **1** and ^{15}N-labelled nornicotine **2** from the commercially available 3-bromopyridine **6**. The formation of the pyrrolidine ring was achieved by reductive aminocyclization of a 1,4-ketoaldehyde with a corresponding amine in the presence of sodium cyanoborohydride. A retrosynthetic analysis of ^{15}N-labelled nicotine **1** is shown in Scheme 2.



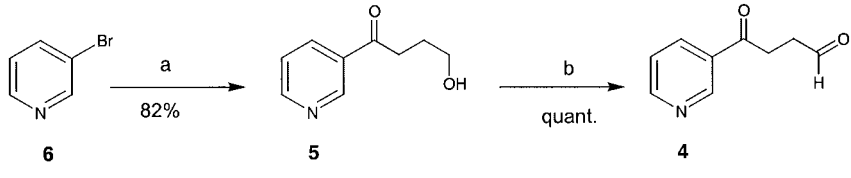
Scheme 2. Retrosynthetic analysis

Our synthesis was initiated by the direct conversion of 3-bromopyridine **6** to the hydroxyketone **5** in 82% yield using halogen–lithium exchange of **6** with *n*-BuLi followed by treatment with γ-butyrolactone.[13] Oxidation of the alcohol **5** in mild conditions using the Swern method[14] led to the ketoaldehyde **4** which was immediately used in the next step without purification (Scheme 3).

With the ketoaldehyde **4** in hand, we attempted the synthesis of *N*-benzylnornicotine **3** by reductive aminocyclization as described in the literature.[15]

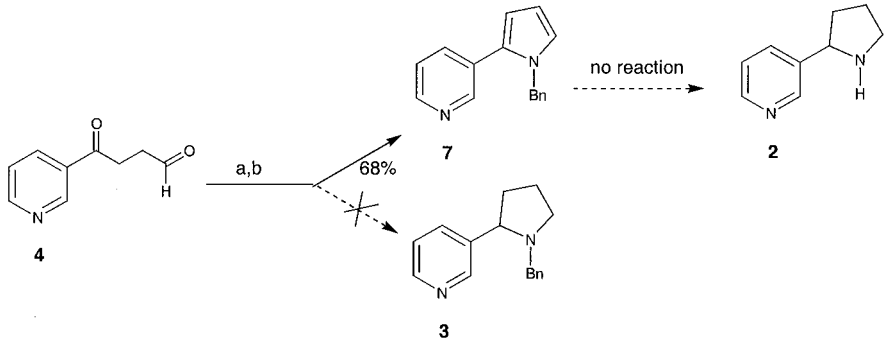
It is well known that benzylamine is exceptionally useful for introducing a nitrogen atom into organic compounds because it is a good nucleophile and because the benzyl group can easily be removed from the product either by catalytic hydrogenation[16] or by the use of sodium in liquid ammonia.[17] A desirable approach for our purpose was, therefore, to use ^{15}N-enriched benzylamine as a sole nitrogen source.

^{15}N-enriched benzylamine is easily prepared in high yield either from potassium ^{15}N-enriched phthalimide and benzyl chloride or from ^{15}N-enriched ammonia as reported in the literature.[18,19] In our hands, however, treating the ketoaldehyde **4** with benzylamine in the presence



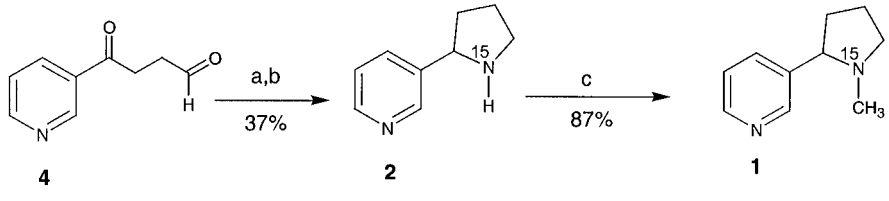
Scheme 3. Reagents and conditions: (a) BuLi, −100°C, THF-hexane then γ-butyrolactone, −80°C to −20°C, ether; (b) $(ClCO)_2$, DMSO, TEA, CH_2Cl_2, −50°C to RT

of sodium cyanoborohydride failed to produce the desired product **3**. Rather, we observed the formation of the oxidized form, pyrrole derivative **7**. Unfortunately, attempts to transform **7** into **2** by catalytic hydrogenation were completely unsuccessful (Scheme 4).



Scheme 4. Reagents and conditions: (a) $BnNH_2$, MeOH, RT; (b) $NaBH_3CN$, RT

Therefore, an alternative route to prepare ^{15}N-labelled nicotine was investigated using ammonia to carry out the reductive aminocyclization. Treatment of **4** with excess ^{15}N-enriched ammonia in the same conditions previously mentioned afforded directly ^{15}N-labelled nornicotine **2**. It should be noted that ^{15}N-enriched ammonia was easily obtained in good yield by treating commercially available ^{15}N-enriched ammonium chloride (10% atom ^{15}N)—as an inexpensive label source—with potassium hydroxide in pellets (Scheme 5).



Scheme 5. Reagents and conditions: (a) $^{15}NH_3$, MeOH, RT; (b) $NaBH_3CN$, RT; (c) HCHO 37%, HCO_2H 98%, 80°C

Conversion of ^{15}N-labelled nornicotine **2** to the ^{15}N-labelled nicotine **1** in 87% yield essentially followed the Eschweiler–Clarke protocol, as reported in the literature.[20] The ^{15}N NMR analysis of **1** and **2** displayed a single peak for ^{15}N at −325.51 and −328.42 ppm, respectively, consistent with the introduction of ^{15}N only in the pyrrolidine ring. Analysis by MS showed mass enhancements of 1 m.u. in **1** and **2**,

indicating the incorporation of only a single ^{15}N per molecule. From the enhancement of the M + 1 peaks, an enrichment of ca. 10% could be deduced, consistent with that in the starting ammonium chloride. EI fragmentation patterns of ^{15}N-**1** and ^{15}N-**2** were consistent with those obtained from authentic **1** and **2**.

Conclusion

In summary, we have described the first synthesis of specifically [^{15}N-*pyrrolidinyl*]-labelled nornicotine **2** and nicotine **1**, important tracer compounds for the study of the metabolism of pyrrolidine alkaloids in plants. Using the synthesis presented, these alkaloids were synthesized in 5 steps for **1** and 4 steps for **2** from 3-bromopyridine in 26% and 30% overall yields, respectively.

Experimental section

Materials and methods

All reagents were purchased from Acros Organics and Aldrich Co. and all anhydrous solvents were prepared in accordance with standard protocols. ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, using $CDCl_3$ as internal standard (7.26 and 77.16 ppm, respectively). ^{15}N NMR spectra were recorded at 30.42 MHz using a 10 mm diameter broad band probe head and $CH_3{}^{15}NO_2$ as internal standard. Flash column chromatography was performed on Merck silica gel (60 Å, 230–400 mesh).

4-Hydroxy-1-pyridin-3-yl-butan-1-one (5)

To a solution of *n*-butyllithium (2.5 M,. 13.6 mL, 34 mmol) in THF at −100°C was slowly added a solution of 3-bromopyridine (4.9 g, 31 mmol) in THF (15 mL). During this addition, the temperature was kept carefully at ca. −100°C. After stirring for 5 min, a solution of γ-butyrolactone (2.4 g, 27 mmol) in Et_2O (20 mL) was added. The reaction mixture was stirred for 30 min at −100°C, then allowed to warm to 0°C and quenched with saturated aqueous NH_4Cl (20 mL). The organic phase was extracted with Et_2O (3 × 40 mL), dried over $MgSO_4$, filtered

and evaporated *in vacuo*. Purification by flash chromatography (AcOEt 100%) gave the alcohol **5** (3.65 g, 82%) as a yellow oil. ^{1}H NMR δ (ppm) 2.01–2.08 (m, 2H), 2.50 (s broad, OH), 3.13–3.20 (t, 2H, $J = 7.0$ Hz), 3.73–3.80 (t, 2H, $J = 7.0$ Hz), 7.40–7.50 (m, 1H), 8.24–8.28 (m, 1H), 8.75–8.78 (m, 1H), 9.17–9.19 (m, 1H); ^{13}C NMR δ (ppm) 26.7, 35.5, 61.8, 123.7, 132.2, 135.5, 149.5, 153.4, 199.2.

4-Oxo-4-pyridin-3-yl-butyraldehyde (4)

To a solution of oxalyl chloride (350 µL, 3.7 mmol) in CH_2Cl_2 (5 mL) at -50°C was slowly added a solution of DMSO (550 µL, 7.5 mmol) in CH_2Cl_2 (3 mL). After stirring for 2 min, a solution of alcohol **5** (560 mg, 3.4 mmol) in CH_2Cl_2 (5 mL) was added. Stirring was continued for an additional 15 min. TEA (2.4 mL, 17 mmol) was added and the reaction mixture was stirred for 10 min and then allowed to warm to room temperature. Water (15 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3×30 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated *in vacuo* to afford the crude aldehyde **4** as a yellow oil. The product was immediately used in the next step without purification. ^{1}H NMR δ (ppm) 2.94–3.0 (t, 2H, $J = 6.4$ Hz), 3.26–3.32 (t, 2H, $J = 6.4$ Hz), 7.37–7.43 (m, 1H), 8.20–8.23 (m, 1H), 8.74–8.76 (m, 1H), 9.1 (s, 1H), 9.86 (s, 1H). ^{13}C NMR δ (ppm): 31.0, 37.8, 123.5, 132.6, 135.9, 149.4, 153.7, 196.2, 201.2.

3-Pyrrolidin-2-yl-pyridine, ^{15}N-labelled nornicotine (2)

To a solution of ketoaldehyde **4** (391 mg, 2.4 mmol) in MeOH (20 mL) was added a solution of ammonium-^{15}N-labelled hydroxide (3 N, 8 mL, 24 mmol). After 20 min of stirring at room temperature, $NaBH_3CN$ (302 mg, 4.8 mmol) was added and the pH adjusted to ca. 6–8 with conc. HCl. The reaction mixture was stirred for 16 h and then made alkaline by the addition of solid K_2CO_3 to saturation. The paste obtained was extracted with CH_2Cl_2 (5×30 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated *in vacuo*. Purification by flash chromatography (CH_2Cl_2/MeOH/NH_3 = 75/25/1) gave ^{15}N-labelled nornicotine **2** as a slightly yellow oil (132 mg, 37% from alcohol **5**). ^{1}H NMR δ (ppm) 1.53–1.70 (m, 1H), 1.82–1.92 (m, 2H), 2.0–2.2 (m, 1H), 2.3 (s, 1H), 2.90–3.18 (m, 2H), 4.08 (t, 1H, $J = 7.6$ Hz), 7.13–7.20 (dd, 1H, $J = 7.9$ Hz, $J = 4.9$ Hz), 7.61–7.66 (pseudo dt, 1H, $J = 7.9$ Hz, $J = 2.1$ Hz), 8.38–8.41 (dd, 1H, $J = 4.9$ Hz, $J = 1.7$ Hz,), 8.51

(d, 1H, $J = 1.7$ Hz); ^{13}C NMR δ (ppm) 25.3, 34.1, 46.7, 59.8, 123.2, 134.0, 140.0, 148.0, 148.4; ^{15}N NMR δ (ppm) -328.42 (N pyrrolidine), -72.02 (N pyridine); MS (EI) m/z 148 (M-H), 149 (M^+).

3-(1-Methylpyrrolidin-2-yl)-pyridine, ^{15}N-labelled nicotine (1)

To ^{15}N-labelled nornicotine **2** (217 mg, 1.47 mmol) in 98% formic acid (3.3 mL) was added 37% aqueous formaldehyde (1.7 mL). The solution was heated with stirring for 3 h at 80°C. After being cooled to 25°C, the mixture was made alkaline, saturated with solid K_2CO_3 and extracted with CH_2Cl_2 (5×30 mL). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated *in vacuo*. Purification by flash chromatography (CH_2Cl_2/MeOH = 98/2) afforded ^{15}N-labelled nicotine **1** (208 mg, 87%) as a pale-yellow oil. ^{1}H NMR δ (ppm) 1.62–2.10 (m, 3H), 2.14 (s, 3H), 2.17–2.36 (m, 2H), 3.07 (t, 1H, $J = 8.3$ Hz), 3.23 (t, 1H, $J = 8.3$ Hz), 7.24 (dd, 1H, $J = 8.0$ Hz, $J = 4.9$ Hz), 7.71 (dt, 1H, $J = 8.0$ Hz, $J = 2.0$ Hz), 8.47 (dd, 1H, $J = 2.0$ Hz, $J = 4.9$ Hz), 8.51 (t, 1H, $J = 2$ Hz); ^{13}C NMR δ (ppm) 22.7, 35.2, 40.4, 57.1, 69.0, 123.7, 135.0, 138.7, 148.7, 149.6; ^{15}N NMR δ (ppm) -325.51 (N pyrrolidine); MS (EI) m/z 162 (M-H), 163 (M^+).

3-(1-Benzyl-1H-pyrrol-2-yl)-pyridine (7)

This compound was prepared by a procedure analogous to that employed for the preparation of **2**. Benzylamine was substituted for ammonium-^{15}N-labelled hydroxide. Purification by flash chromatography (AcOEt/Hexane = 2/8) gave **7** as a yellow oil. ^{1}H NMR δ (ppm) 5.02 (s, 2H), 6.18–6.25 (m, 2H), 6.70–6.72 (m, 1H), 6.83–6.88 (m, 1H), 7.10–7.17 (m, 5H), 7.42–7.48 (m, 1H), 7.51 (t; 1H, $J = 2$ Hz), 8.47 (dd, 1H, $J = 4.9$ Hz, $J = 2.0$ Hz); ^{13}C NMR δ (ppm) 50.9, 108.9, 110.3, 123.2, 124.2, 126.2, 127.6, 128.9, 131.5, 135.8, 138.3, 148.1, 149.6.

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