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Synthesis of four racemic nicotine isotopomers doubly labelled with stable isotope

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Four racemic nicotine isotopomers, doubly labelled with stable isotope, were prepared for use in studies of plant metabolism. First, starting from halogeno nicotinates, one deuterium was introduced into the pyridine moiety by reductive dehalogenation with zinc, and choosing deuterated acetic acid as the acidic medium. Pyridine ^{2}H-labelled nornicotine derivatives were then formed in two reaction steps, a condensation reaction with *N*-vinylpyrrolidinone followed by catalytic hydrogenation. The deuteromethylation of these monodeuterated nornicotines was achieved by reduction of the corresponding ethyl carbamates with deuterated lithium aluminium hydride. The four regioisotopomers obtained were at the least 90% labelled on each site, sufficient for the metabolic studies envisaged.

Keywords: deuterated pyridine; *N*-^{2}H-methylation; ^{2}H-labelled compounds; nicotine

Introduction

Some natural compounds are characterized by contradictory pharmacological profiles. This is the case of nicotine **1** (Figure 1), one of the major alkaloids present in tobacco. It is well known that tobacco smoking induces physical and psychological addictions associated with health risks, such as cancer and cardiovascular diseases. In contrast, since the 1980s, several epidemiological studies have shown that people with a history of cigarette smoking have lower rates of neurodegenerative disorders such as Alzheimer and Parkinson diseases.[1] It is now well documented that neuronal nicotinic acetylcholine receptors (nAChRs) are implicated in these diseases and therefore are clearly potential targets for the treatment of such disorders.[2] Furthermore, the modulatory input of specific nAChR subtypes to other important neurotransmitters such as dopamine has been demonstrated.[3]

Although a lot of synthetic ligands of the nAChRs have been designed and synthesized in the last years,[4] it has to be taken into account that nicotine metabolites or other constituents of tobacco such as cotinine **2**, nornicotine **3**[5] or metanicotine **4**[6] (Figure 1), also bind to nicotinic central nervous system receptors. Some of these compounds such as cotinine **2** (Figure 1) have distinctive receptors in mammal tissues and bind with higher affinity to these proteins than does nicotine.[7]

That nicotine turnover occurs in natural systems has been recognized for many years but the lifetimes and roles of its catabolic products in plants and human still remain to be elucidated. In plants, *N*-demethylation of nicotine **1** to nornicotine **3** is a key step in the biosynthesis of other bioactive molecules such as the *N*-acylnornicotines implicated for example in the defence-response.[8] However, nicotine degradation is not only confined to plants. Several degradation pathways of nicotine have been described for micro-organisms including a fungal route, initiated by a *N*-demethylation step.[9]

To elucidate the mechanisms of these demethylative or oxidative reactions of nicotine and related alkaloids, a number of studies using isotopically labelled nicotine analogues have been carried out.[10a] A detailed understanding of the mechanisms involved in degradation steps can bring crucial information on the transition states derived from the substrate in these enzymatic reactions. This in turn can highlight similarities and differences between the mechanisms, understanding that it can aid in the conception and synthesis of novel molecules mimicking transition states. Those could be considered as potential pharmacological tools or therapeutic agents susceptible either to block the enzymatic metabolic pathway or to bind to the targeted receptor.

Taking into account that enzyme reaction kinetics are more or less sensitive to the presence or absence of heavy isotopes, kinetic isotope effect (KIE)[11] studies provide a powerful way to access enzymatic mechanisms. Thus, *N*-[$^{2}H_3$-*methyl*]nicotine was used to define the mechanism of *N*-demethylation in cell suspension cultures of *Nicotiana*[10a]

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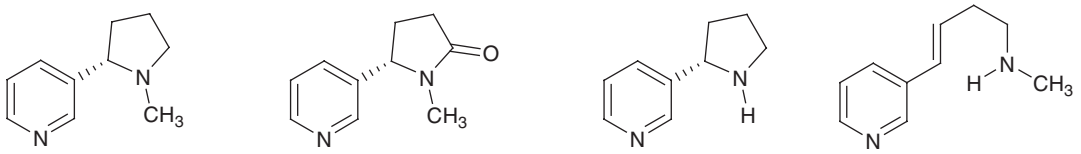


Figure 1. Nicotine and related metabolites.

while [^{13}C, $^{2}H_3$-methyl]nicotine and [1′-^{15}N]nicotine and nornicotine[12] have facilitated the elucidation of the pathway of nicotine degradation in these cultures.[13] In order to further probe nicotine degradative enzymatic reaction mechanisms by use of the associated KIE effects, we have prepared nicotine doubly labelled on the pyridine ring and the *N*-methyl of the pyrrolidine substituent. Such doubly labelled species provide an internal reference peak from the ^{2}H aromatic signal, allowing better precision in determining the ($^{2}H/^{1}H$) ratio in the methyl group than can be obtained using an internal standard (e.g. dioxane-d_8). Hence, measurement of kinetic changes in the ($^{2}H/^{1}H$) ratios and calculation of kinetic effects may be performed *in vivo* by ^{2}H qNMR of GC-MS, under quantitative conditions.

Results and discussion

Convenient synthetic routes to nicotine ^{2}H-labelled on the pyridine[14] or the pyrrolidine[15,16] moieties have been published, but to our knowledge no synthesis of specifically *N*-C^2H_3 and ^{2}H-aromatic doubly labelled nicotine species **5** (Scheme 1) have been up to date reported in the literature. In this paper, we describe the preparation of four ^{2}H doubly labelled nicotines **5a–d** from commercially available nicotinates **6a–d**. The strategy adopted, as outlined in the retrosynthetic pathway depicted in Scheme 1, was to incorporate a deuterium atom into one of four positions in the pyridine ring and the three others into the *N*-methyl substituent of the pyrrolidine ring.

Preparation of pyridine labelled myosmines 8

The one-pot synthesis of myosmine earlier described by Jacob[17] and efficiently applied by us in the synthesis of racemic nornicotine and nicotine[10b] appeared to be the strategy of choice to access ^{2}H-myosmine analogues **8**, which after reduction could be doubly labelled using a *N*-methylation procedure with deuterated reagents (Scheme 2).

Starting from commercially available halogeno nicotinates **6a–d**, the deuterated nicotinate regioisomers **7a–d** were formed in good yields (yields: 51–83%) by reduction in the halogeno nicotinates **6** using zinc metal and [$^{2}H_4$]-acetic acid in dry diethyl ether. These deuterated intermediates **7** were then reacted with *N*-vinylpyrrolidinone in the presence of sodium hydride in refluxed THF for 1 h. The expected deuterated myosmines **8a–d** were formed in a one-pot/two-step manner by harsh acidic hydrolysis–decarboxylation procedure in concentrated HCl, followed by a basic work-up with concentrated aqueous NaOH solution. After purification by silica gel chromatography **8a–d** were obtained with moderate but not optimized yields (yields: 37–73%). It is to be noted that starting from **6d**, direct formation of 6-chloro myosmine using Jacob's procedure failed, because hard aqueous basic treatment systematically afforded the 6-OH myosmine derivative. Reduction in the imine function was carried on by hydrogenation in the presence of a catalytic amount of Pd/C (10%) to give the monodeuterated nornicotines **9a–d** (yields: 65–96%, Scheme 2).

Preparation of ^{2}H-*N*-1′-C^2H_3 nicotines 5

As has previously been found, *N*-methylation of nicotine with iodomethane furnished a complex mixture of *N*-1 or *N*-1′ nicotinium iodides. An efficient solution was to deprotonate nornicotine in the presence of *n*BuLi followed by *N*-alkylation with iodomethane.[18] Another simple alternative to introduce an *N*-1′-CH_3 on the nornicotine was described by us for the synthesis of the non-natural (*R*)-nicotine isomer[19] and applied here for the synthesis of racemic deuterated nicotines using a reduction step with deuteride (Scheme 3).

Following acylation of nornicotines **9a–d** with ethyl chloroformate in the presence of K_2CO_3, the *N*-ethylcarbamates **10a–d** (yields: 78% to quantitative) were reduced with an excess of deuterated lithium aluminium hydride and the reaction mixture hydrolysed with D_2O. The expected racemic doubly labelled nicotines **5a–d** were purified by chromatography on silica gel and isolated as colourless oils (yields: 57–73%). ^{1}H and ^{2}H-NMR spectra of the four doubly labelled nicotines **5a–d** were analysed. Regioselective deuterium incorporation on the pyridine ring was quantified by ^{1}H NMR study and the *N*-C^2H_3 percentage incorporation was calculated from ^{2}H NMR spectra using the *N*-1′-C^2H_3/pyridine C^2H ratio. It was found that regioselective deuterium incorporation on the pyridine ring was up to 90% (90–93%) and *N*-1′-C^2H_3 pyrrolidine deuteration ranged between 92 and 100%.

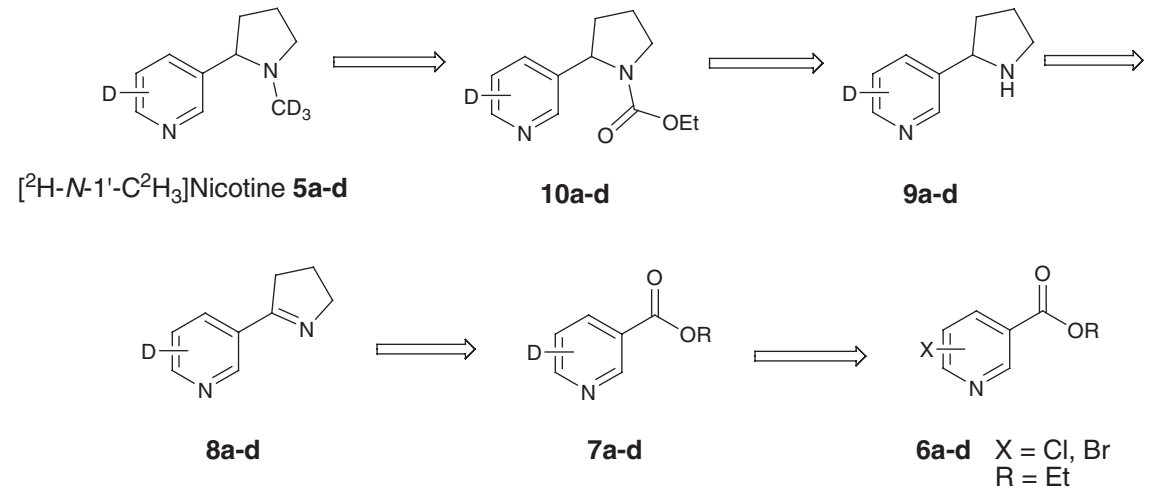
Experimental

General

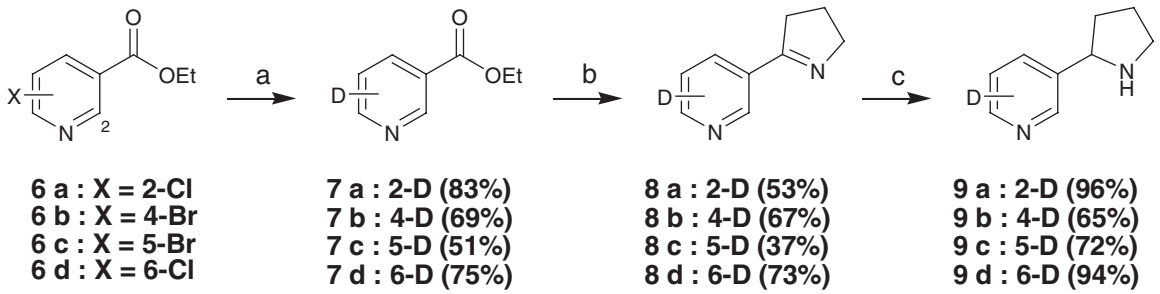
The nicotinates were purchased from Acros Organics or Aldrich Chemical. CD_3CO_2D (98 atom % D) and $LiAlD_4$ (98 atom % D) were purchased from Acros Organics. All reactions were monitored by TLC (Kieselgel $60F_{254}$ MERCK aluminium sheet) with detection by UV light and/or with ethanolic phosphomolybdic acid solution. Flash column chromatography was performed on silica gel 60 ACC 40–63 μm (Carbo-erba reagents – SDS). ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a BRUKER Avance 300, in an appropriate solvent (Me_4Si as an internal standard for $CDCl_3$). ^{2}H NMR spectrum was recorded on a BRUKER Avance DP × 400 at 61 MHz in CH_2Cl_2 (internal reference). Mass spectroscopy was registered on a Hewlett Packard 5989A (EI, 70 eV).

[6-^{2}H]ethylnicotinate 7d

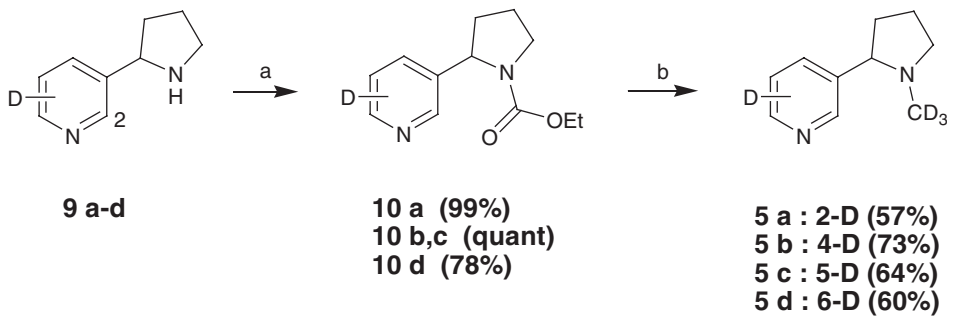
To a stirred solution of ethyl 6-chloronicotinate **6d** (2.7 g, 14.5 mmol, 1 equiv.) in Et_2O (20 mL) were added zinc dust



Scheme 1. Retrosynthetic analysis.



Scheme 2. Reagents and conditions: (a) Zn dust, CD_3COOD, Et_2O then D_2O; (b) (i) *N*-vinylpyrrolidinone, NaH, THF, reflux, 1 h; (ii) conc. aqueous HCl, H_2O, reflux, overnight; (iii) conc. aqueous NaOH; and (c) Pd/C (10%), H_2, MeOH.



Scheme 3. Reagents and conditions: (a) EtOCOCl, K_2CO_3, DCM; and (b) $LiAlD_4$, THF, 3 h.

(4.55 g, 72.5 mmol, 5 equiv.) and CD_3CO_2D (3.4 mL, 29.3 mmol, 4 equiv.). The mixture was stirred for 3 h at room temperature. The orange mixture was then made neutral with a saturated aqueous K_2CO_3 solution and the zinc was filtered off. The filtrate was then basified (pH 14) and the aqueous layer was extracted with Et_2O (3 × 40 mL, dried over $MgSO_4$ filtered and concentrated under reduced pressure. The crude product was then purified by chromatography on silica gel eluting with DCM/Et_2O (98/2) to afford **7d** as a colourless oil (1.6 g, 10.9 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (t, 3H, J = 7.2 Hz), 4.39 (q, 2H, J = 7.2 Hz), 7.36 (d, 1H), 8.27 (dd, 1H), 9.20 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2 (CH_3), 61.4 (CH_2), 123.0 (CH_{ar}), 126.3 (C^{IV}), 137.0 (CH_{ar}), 150.8 (CH_{ar}), 152.5 (CH_{ar}), 165.2 (C = O); MS (EI) *m/e* 152 ($M^{+\cdot}$, 37), 124 (51), 107 (100), 79 (65), 51 (25).

[2-^{2}H]ethylnicotinate 7a

Following the protocol for the synthesis of **7d**, 2-deuterated ethyl nicotinate **7a** was obtained as oil (yield: 83%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (t, 3H, J = 7.2 Hz, H_9), 4.42 (q, 2H, J = 7.2 Hz, H_8), 7.40 (dd, 1H, J = 5.0 Hz, J = 8 Hz, H_5), 8.31 (dd, 1H, J = 1.8 Hz, J = 8.0 Hz, H_4), 8.78 (dd, 1H, J = 1.8 Hz, J = 5.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2 (CH_3, C_9), 61.4 (CH_2, C_8), 123.2 (CH_{ar}, C_5), 126.2 (C^{IV}, C_3), 136.9 (CH_{ar}, C_4), 150.5 (CH_{ar}, C_2), 153.2 (CH_{ar}, C_6), 165.2 (C = O, C_7).

[4-^{2}H]ethylnicotinate 7b

Following the protocol for the synthesis of **7d**, 4-deuterated ethyl nicotinate **7b** was obtained as oil (yield: 69%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (t, 3H, J = 7.1 Hz, H_9), 4.42 (q, 2H, J = 7.1 Hz, H_8), 7.41 (d, 1H, J = 4.6 Hz, H_5), 8.79 (d, 1H, J = 4.6 Hz, H_6), 9.23 (d, 1H, J = 0.8 Hz, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2 (CH_3, C_9), 61.5 (CH_2, C_8), 123.2 (CH_{ar}, C_5), 126.4 (C^{IV}, C_3), 136.9 (CD, J = 23.8 Hz, C_4), 150.7 (CH_{ar}, C_2), 153.1 (CH_{ar}, C_6), 165.2 (C = O, C_7); MS (EI) *m/e* 152 (M^+, 29), 124 (53), 107 (100), 79 (72), 52 (45), 49 (47), 41 (49).

[5-^{2}H]ethylnicotinate 7c

Following the protocol for the synthesis of **7d**, 5-deuterated ethyl nicotinate **7c** was obtained as oil (yield: 51%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (t, 3H, J = 7.2 Hz, H_9), 4.42 (q, 2H, J = 7.2 Hz, H_8), 8.31 (bs, 1H, H_4), 8.77 (bs, 1H, H_6), 9.23 (bs, 1H, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8 (CH_3, C_9), 60.9 (CH_2, C_8), 122.5 (CH_{ar}, C_5), 125.9 (C^{IV}, C_3), 136.4 (CH_{ar}, C_4), 150.4 (CH_{ar}, C_2), 152.8 (CH_{ar}, C_6), 164.7 (C = O, C_7); MS (EI) *m/e* 152 (M^+, 34), 124 (55), 107 (100), 79 (66), 52 (37).

[6-^{2}H]myosmine 8d

Sodium hydride (950 mg of a 60% dispersion in mineral oil, 14.2 mmol, 1.3 equiv.) was washed free of mineral oil with petroleum ether (3 × 20 mL). The flask was fitted with a reflux condenser, flushed with argon, and charged with freshly distilled anhydrous THF (20 mL). A solution of ethyl 6-deuteronicotinate **7d** (1.66 g, 10.9 mmol, 1 equiv.) and *N*-vinylpyrrolidinone (1.3 mL, 12 mmol, 1.1 equiv.) in THF (6 mL) was added in one portion. The mixture was stirred magnetically, and heated to 35°C, until no more gas evolution. Then the mixture was heated to reflux for 90 min and cooled to room temperature. Concentrated HCl (6 mL), diluted with H_2O (10 mL), was added, and the THF was removed on a rotary evaporator. Additional concentrated HCl (10 mL) and H_2O (20 mL) were added, and the mixture was heated to reflux overnight. The solution was made basic with concentrated aqueous NaOH, and the aqueous layer was extracted with methylene chloride (3 × 40 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (DCM then DCM/MeOH 98/2) to give **8d** as a pale yellow solid (402 mg, 10.3 mmol, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.0–2.13 (m, 2H, $H_{4'}$), 2.9–3.0 (t, 2H, J = 7.3 Hz, $H_{3'}$), 4.03–4.13 (t, 2H, J = 7.3 Hz, $H_{5'}$), 7.34 (d, 1H, J = 8.0 Hz, H_5), 8.18 (dd, 1H, J = 8.0 Hz, J = 1.9 Hz, H_4), 9.0 (d, 1H, J = 1.9 Hz, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5 (CH_2, $C_{4'}$), 37.7 (CH_2, $C_{3'}$), 61.6 (CH_2, $C_{5'}$), 123.2 (CH_{ar}, C_5), 130.2 (C^{IV}, C_3), 134.6 (CH_{ar}, C_4), 149.0 (CH_{ar}, C_2), 150.8 (C–D, J = 27.2 Hz, C_6), 171.0 (C = N, $C_{2'}$).

[2-^{2}H]myosmine 8a

Following the protocol for the synthesis of **8d**, myosmine **8a** was obtained as a solid (yield: 53%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03–2.13 (m, 2H, $H_{4'}$), 2.92–3.04 (m, 2H, $H_{3'}$) = 4.06–4.15 (m, 2H, $H_{5'}$), 7.36 (dd, 1H, J = 4.8 Hz, J = 7.9 Hz, H_5), 8.19 (d, 1H, J = 7.9 Hz, H_4), 8.66 (d, 1H, J = 4.8 Hz, H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5 (CH_2, $C_{4'}$), 34.7 (CH_2, $C_{3'}$), 61.6 (CH_2, $C_{5'}$), 123.4 (CH_{ar}, C_5), 130.1 (C^{IV}, C_3), 134.6 (CH_{ar}, C_4), 148.7 (CD, J = 27.0 Hz, C_2), 151.1 (CH_{ar}, C_6), 171.0 (C = N, $C_{2'}$); MS (EI) *m/e* 147 (M^+, 86), 146 (79), 119 (100), 106 (25), 79 (22), 42 (17).

[4-^{2}H]myosmine 8b

Following the protocol for the synthesis of **8d**, myosmine **8b** was obtained as a solid (yield: 67%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02–2.14 (m, 2H, $H_{4'}$), 2.92–3.02 (m, 2H, $H_{3'}$), 4.05–4.13 (m, 2H, $H_{5'}$), 7.36 (d, 1H, J = 4.6 Hz, H_5), 8.66 (d, 1H, J = 4.6 Hz, H_6), 9.00 (bs, 1H, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5 (CH_2, $C_{4'}$), 34.8 (CH_2, $C_{3'}$), 61.7 (CH_2, $C_{5'}$), 123.3 (CH_{ar}, C_5), 130.1 (C^{IV}, C_3), 134.5 (CD, J = 24.0 Hz, C_4), 149.1 (CH_{ar}, C_2), 151.2 (CH_{ar}, C_6), 171.0 (C = N, $C_{2'}$); MS (EI) *m/e* 147 (M^+, 75), 146 (75), 119 (100), 106 (24), 79 (23), 42 (18), 41 (18).

[5-^{2}H]myosmine 8c

Following the protocol for the synthesis of **8d**, myosmine **8c** was obtained as a solid (yield: 37%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03–2.13 (m, 2H, $H_{4'}$), 2.95–3.00 (m, 2H, $H_{3'}$), 4.07–4.12 (m, 2H, $H_{5'}$), 8.21 (bs, 1H, H_4), 8.66 (bs, 1H, H_6), 9.01 (d, 1H, J = 1.9 Hz, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5 (CH_2, $C_{4'}$), 29.6 (CH_2, $C_{3'}$), 61.6 (CH_2, $C_{5'}$), 123.1 (CD, J = 24.4 Hz, C_5), 130.2 (C^{IV}, C_3), 134.5 (CH_{ar}, C_4), 149.1 (CH_{ar}, C_2), 151.1 (CH_{ar}, C_6), 171.0 (C = N, $C_{2'}$), MS (EI) *m/e* 147 (M^+, 86), 146 (79), 119 (100), 106 (25), 79 (22), 42 (17).

[6-^{2}H]nornicotine 9d

To a solution of **8d** (822 mg, 5.6 mmol) in degassed MeOH (15 mL) palladium on activated carbon (10% Pd/C) (82 mg, 10% w/w) was added under an argon atmosphere. Argon was replaced with H_2 atmosphere. The mixture was stirred overnight then filtered through celite and the filtrate was evaporated under reduced pressure. The crude product was then purified by chromatography on silica gel (DCM then DCM/MeOH 9/1) to give **9d** (780 mg, 5.26 mmol, 94%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59–1.72 (m, 1H, $H_{4'}$), 1.79–2.01 (m, 2H, $H_{4'}$ + $H_{3'}$), 2.15–2.29 (m, 1H, $H_{3'}$), 2.90–3.10 (m, 1H, $H_{5'}$), 3.14–3.26 (m, 1H, $H_{5'}$), 4.15 (t, 1H, J = 7.7 Hz, $H_{2'}$), 7.24 (d, 1H, 7.8 Hz, H_5), 7.72 (d, 1H, J = 7.8 Hz, H_5), 8.58 (bs, 1H, H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4 (CH_2, $C_{5'}$), 34.3 (CH_2, $C_{3'}$), 46.9 (CH_2, $C_{4'}$), 60.0 (CH, $C_{2'}$), 123.1 (CH_{ar}, C_5), 133.9 (CH_{ar}, C_4), 140.1 (C^{IV}, C_3), 147.8 (CD, J = 26.6 Hz, C_6), 148.5 (CH_{ar}, C_2); MS (EI) *m/e* 149 (M^+, 23), 148 (36), 119 (25), 120 (100), 121 (23), 81 (29), 70 (94).

[2-^{2}H]nornicotine 9a

Following the protocol for the synthesis of **9d**, nornicotine **9a** was obtained as a solid (yield: 96%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60–1.74 (m, 1H, $H_{4'}$), 1.79–2.01 (m, 2H, $H_{4'}$+$H_{3'}$), 2.06 (bs, 1H, $NH_{1'}$), 2.16–2.29 (m, 1H, $H_{3'}$), 3.00–3.10 (m, 1H, $H_{5'}$), 3.16–3.26 (m, 1H, $H_{5'}$), 4.16 (t, 1H, J = 7.6 Hz, $H_{2'}$), 7.24 (dd, 1H, J = 4.8 Hz, J = 7.9 Hz, H_4), 7.71 (dd, 1H, J = 1.5 Hz, J = 7.9 Hz, H_5), 8.46 (dd, 1H, J = 1.5 Hz, J = 4.8 Hz, H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.5 (CH_2, $C_{5'}$), 34.3 (CH_2, $C_{3'}$), 46.9 (CH_2, $C_{4'}$), 60.0 (CH, $C_{2'}$), 123.3 (CH_{ar}, C_5), 134.0 (CH_{ar}, C_4), 140.1 (C^{IV}, C_3), 148.2 (CH_{ar}, C_6), 148.2 (CD, J = 27.0 Hz, C_2); MS (EI) *m/e* 149 (M^+, 23), 148 (31), 121 (24), 120 (68), 119 (45), 81 (37), 70 (100).

[4-^{2}H]nornicotine 9b

Following the protocol for the synthesis of **9d**, nornicotine **9b** was obtained as a solid (yield: 65%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59–1.72 (m, 1H, $H_{4'}$), 1.79–2.01 (m, 2H, $H_{4'}$+$H_{3'}$), 2.15–2.29 (m, 1H, $H_{3'}$), 2.42 (bs, 1H, $NH_{1'}$), 2.90–3.10 (m, 1H, $H_{5'}$), 3.14–3.26 (m, 1H, $H_{5'}$), 4.15 (t, 1H, J = 7.7 Hz, $H_{2'}$), 7.24 (d, 1H, J = 4.5 Hz, H_5), 8.47 (d, 1H, J = 4.5 Hz, H_6), 8.59 (bs, 1H, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4 (CH_2, $C_{5'}$),

34.2 (CH_2, $C_{3'}$), 46.8 (CH_2, $C_{4'}$), 59.9 (CH, $C_{2'}$), 123.0 (CD, $J = 24.0$ Hz, C_5), 133.9 (CH_{ar}, C_4), 140.1 (C^{IV}, C_3), 148.0 (CH_{ar}, C_6), 148.4 (CH_{ar}, C_2) MS (EI) *m/e* 149 (M^+, 18), 148 (33), 121 (28), 120 (87), 119 (51), 107 (37), 94 (28), 70 (100), 30 (46).

[5-^{2}H]nornicotine 9c

Following the protocol for the synthesis of **9d**, nornicotine **9c** was obtained as a solid (yield: 72%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59–1.72 (m, 1H, $H_{4'}$), 1.79–2.01 (m, 2H, $H_{4'}+H_{3'}$), 2.15–2.29 (m, 1H, $H_{3'}$), 2.42 (bs, 1H, $NH_{1'}$), 2.90–3.10 (m, 1H, $H_{5'}$), 3.14–3.26 (m, 1H, $H_{5'}$), 4.15 (t, 1H, $J = 7.7$ Hz, $H_{2'}$), 7.71 (bs, 1H, H_4), 8.47 (bs, 1H, H_6), 8.58 (d, 1H, $J = 2.1$ Hz, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4 (CH_2, $C_{5'}$), 34.2 (CH_2, $C_{3'}$), 46.8 (CH_2, $C_{4'}$), 59.9 (CH, $C_{2'}$), 123.0 (CD, $J = 24.0$ Hz, C_5), 133.9 (CH_{ar}, C_4), 140.1 (C^{IV}, C_3), 148.0 (CH_{ar}, C_6), 148.4 (CH_{ar}, C_2); MS (EI) *m/e* 149 (M^+, 22), 148 (33), 119 (21), 120 (84), 121 (24), 81 (28), 70 (100).

[6-^{2}H]-3-(1′-ethoxycarbonylpyrrolidin-2′-yl)-pyridine 10d

To a solution of **9d** (400 mg, 2.68 mmol, 1 equiv.) in anhydrous DCM (20 mL) anhydrous K_2CO_3 (371 mg, 2.68 mmol, 1 equiv.) was added under argon atmosphere. Then the mixture was cooled to 0°C and EtOCOCl (0.31 mL, 3.22 mmol, 1.2 equiv.) was added dropwise. The mixture was stirred at room temperature for 1 h (monitored by TLC). K_2CO_3 was removed by filtration and the filtrate was evaporated under reduced pressure. The crude product was then purified by chromatography on silica gel (eluent: DCM then DCM Et_2O 8/2) to give **10d** as a colourless oil (465 mg, 2.09 mmol, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93–1.17 (m, 1H, $H_{4'}$), 1.20–1.33 (m, 1H, $H_{4'}$), 1.80–2.00 (m, 4H, $3H_{8'}+H_{4'}$), 2.28–2.47 (m, 1H, $H_{3'}$), 3.54–3.74 (m, 2H, $2H_{7'}$), 3.92–4.05 (m, 1H, $H_{5'}$), 4.05–4.19 (m, 1H, $H_{5'}$), 4.83–5.03 (m, 1H, $H_{2'}$), 7.24 (d, 1H, $J = 7.8$ Hz, H_5), 7.45–7.56 (bs, 1H, H_4), 8.5 (bs, 1H, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3 & 14.6 (CH_3, $C_{8'}$), 22.9 & 23.5 (CH_2, $C_{4'}$), 34.4 & 35.4 (CH_2, $C_{3'}$), 46.9 & 47.2 (CH_2, $C_{5'}$), 58.7 & 60.0 (CH_2, $C_{7'}$), 60.9 (CH, $C_{2'}$), 123.0 (CH, C_5), 132.9 & 133.1 (CH, C_4), 138.9 & 139.4 (C^{IV}, C_3), 147.5 (CD or CH, C_6 or C_2), 148.0 (CH or CD, C_2 or C_6), 155.0 (C = O, $C_{6'}$); MS (EI) *m/e* 221 ($M^{+\cdot}$, 29), 192 (100), 148 (59), 142 (49), 70 (68), 29 (43).

[2-^{2}H]-3-(1′-ethoxycarbonylpyrrolidin-2′-yl)-pyridine 10a

Following the protocol for the synthesis of **10d**, compound **10a** was obtained as oil (yield: 99%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93–1.17 (m, 1H, $H_{4'}$), 1.20–1.35 (m, 1H, $H_{4'}$), 1.80–2.00 (m, 4H, $3H_{8'}+H_{4'}$), 2.28–2.47 (m, 1H, $H_{3'}$), 3.54–3.74 (m, 2H, $2H_{7'}$), 3.92–4.05 (m, 1H, $H_{5'}$), 4.05–4.19 (m, 1H, $H_{5'}$), 4.83–5.03 (m, 1H, $H_{2'}$), 7.18–7.28 (bs, 1H, H_5), 7.45–7.57 (bs, 1H, H_4), 8.39–8.55 (bs, 1H, H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4 & 14.6 (CH_3, $C_{8'}$), 23.0 & 23.6 (CH_2, $C_{4'}$), 34.5 & 35.5 (CH_2, $C_{3'}$), 47.0 & 47.3 (CH_2, $C_{5'}$), 58.8 & 59.0 (CH_2, $C_{7'}$), 61.1 (CH, $C_{2'}$), 123.3 (CH_{ar}, C_5), 133.0 & 133.4 (CH_{ar}, C_4), 139.5 (C^{IV}, C_3), 147.7 (CD, C_2), 148.1 (CH_{ar}, C_6), 155.1 (C = O, $C_{6'}$); MS (EI) *m/e* 221 ($M^{+\cdot}$, 21), 192 (100), 148 (64), 142 (58), 70 (91), 29 (45).

[4-^{2}H]-3-(1′-ethoxycarbonylpyrrolidin-2′-yl)-pyridine 10b

Following the protocol for the synthesis of **10d**, compound **10b** was obtained as oil (yield: quantitative).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93–1.17 (m, 1H, $H_{4'}$), 1.20–1.33 (m, 1H, $H_{4'}$), 1.80–2.00 (m, 4H, $3H_{8'}+H_{4'}$), 2.28–2.47 (m, 1H, $H_{3'}$), 3.54–3.74 (m, 2H, $2H_{7'}$), 3.92–4.05 (m, 1H, $H_{5'}$), 4.05–4.19 (m, 1H, $H_{5'}$), 4.83–5.03 (m, 1H, $H_{2'}$), 7.24 (d, 1H, $J = 4.5$ Hz, H_5), 8.39–8.58 (bs, 2H, H_2+H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4 & 14.7 (CH_3, $C_{8'}$), 23.0 & 23.7 (CH_2, $C_{4'}$), 34.5 & 35.6 (CH_2, $C_{3'}$), 47.0 & 47.4 (CH_2, $C_{5'}$), 58.8 & 59.1 (CH_2, $C_{7'}$), 61.1 (CH, $C_{2'}$), 123.1 (CH, C_5), 132.9 & 133.1 (CD, C_4), 138.9 & 139.5 (C^{IV}, C_3), 147.7 (CH, C_6 or C_2), 148.2 (CH, C_2 or C_6), 155.2 (C = O, $C_{6'}$).

[5-^{2}H]-3-(1′-ethoxycarbonylpyrrolidin-2′-yl)-pyridine 10c

Following the protocol for the synthesis of **10d**, compound **10c** was obtained as oil (yield: quantitative).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93–1.17 (m, 1H, $H_{4'}$), 1.20–1.35 (m, 1H, $H_{4'}$), 1.80–2.00 (m, 4H, $3H_{8'}+H_{4'}$), 2.28–2.47 (m, 1H, $H_{3'}$), 3.54–3.74 (m, 2H, $2H_{7'}$), 3.92–4.05 (m, 1H, $H_{5'}$), 4.05–4.19 (m, 1H, $H_{5'}$), 4.83–5.03 (m, 1H, $H_{2'}$), 7.48 (bs, 1H, H_4), 8.39–8.55 (bs, 2H, H_2+H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5 (CH_3, $C_{8'}$), 23.1 & 23.7 (CH_2, $C_{4'}$), 34.5 & 35.6 (CH_2, $C_{3'}$), 47.4 (CH_2, $C_{5'}$), 58.9 (CH_2, $C_{7'}$), 61.1 (CH, $C_{2'}$), 123.1 (CD, C_5), 132.9 (CD, C_4), 139.0 & 139.6 (C^{IV}, C_3), 147.7 (CH, C_6 or C_2), 148.1 (CH, C_2 or C_6), 155.2 (C = O, $C_{6'}$); MS (EI) *m/e* 221 ($M^{+\cdot}$, 28), 192 (100), 148 (68), 142 (60), 70 (83), 29 (47).

[6-^{2}H-*N*-1′-C^2H_3]nicotine 5d

To a suspension of $LiAlD_4$ (256 mg, 6.1 mmol, 6 equiv.) in dry THF (10 mL) a solution of **10d** (225 mg, 1.02 mmol) in dry THF (5 mL) was added under argon atmosphere. The mixture was stirred for 3 h and then quenched with D_2O (50 mL). DCM was added and the aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were concentrated under reduced pressure. The crude product was then purified by chromatography on silica gel (eluent: DCM then DCM/MeOH 95/5) to give **5d** (100 mg, 0.612 mmol, 60%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65–2.10 (m, 3H, $H_{3'}+2H_{4'}$), 2.16–2.25 (m, 1H, $H_{3'}$), 22.26–2.36 (m, 1H, $H_{5'}$), 3.02–3.12 (m, 1H, $H_{2'}$), 3.20–3.30 (m, 1H, $H_{5'}$), 7.26 (d, 1H, $J = 7.8$ Hz, H_5), 7.70 (dd, 1H, $J = 2$ Hz $J = 7.8$ Hz, H_4), 8.49 (d, 1H, $J = 2$ Hz, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.6 (CH_2, $C_{4'}$), 35.4 (CH_2, $C_{3'}$), 39.8 (CD_3, N–CD_3), 56.9 (CH_2, $C_{5'}$), 68.8 (CH, $C_{2'}$), 123.4 (CH_{ar}, C_5), 134.8 (CH_{ar}, C_4), 138.8 (C^{IV}, C_3), 148.3 (CD, C_6), 149.6 (CH_{ar}, C_2); MS[14] (EI) *m/e* 166 ($M^{+\cdot}$, 13), 165 (12), 137 (18), 87 (100), 45 (14); ^{2}H NMR (61 MHz, CH_2Cl_2) δ 8.46 (1 ^{2}H, $^2H_6$), 2.09 (3 ^{2}H, C^2H_3).

[2-^{2}H-*N*-1′-C^2H_3]nicotine 5a

Following the protocol for the synthesis of **5d**, nicotine **5a** was obtained as oil (yield: 57%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.66–2.05 (m, 3H, $H_{3'}+2H_{4'}$), 2.16–2.26 (m, 1H, $H_{3'}$), 2.26–2.36 (m, 1H, $H_{5'}$), 3.02–3.12 (m, 1H, $H_{2'}$), 3.20–3.30 (m, 1H, $H_{5'}$), 7.28 (dd, 1H, $J = 7.9$ Hz, $J = 4.7$ Hz, H_5), 7.70 (dd, 1H, $J = 1.5$ Hz, $J = 7.8$ Hz, H_4), 8.49 (dd, 1H, $J = 1.5$ Hz, $J = 4.7$ Hz, H_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.6 (CH_2, $C_{4'}$), 35.2 (CH_2, $C_{3'}$), 56.9 (CH_2, $C_{5'}$), 68.8 (CH, $C_{2'}$), 123.6 (CH_{ar}, C_5), 134.9 (CH_{ar}, C_4), 138.6 (C^{IV}, C_3), 148.6 (CH_{ar}, C_6), 149.5 (CD, C_2); MS (EI) *m/e* 166 (M^+, 13), 165 (9), 137 (13), 87 (100), 45 (13); ^{2}H NMR (61 MHz, CH_2Cl_2) δ 8.52 (1 ^{2}H, $^2H_2$), 2.09 (3 ^{2}H, C^2H_3).

[4-^{2}H-*N*-1′-C^2H_3]nicotine 5b

Following the protocol for the synthesis of **5d**, nicotine **5b** was obtained as oil (yield: 73%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.66–2.07 (m, 3H, $H_{3'}+2H_{4'}$), 2.16–2.26 (m, 1H, $H_{3'}$), 2.26–2.36 (m, 1H, $H_{5'}$), 3.02–3.12 (m, 1H, $H_{2'}$), 3.20–3.30 (m, 1H, $H_{5'}$), 7.25 (d, 1H, $J = 4.7$ Hz, H_5), 8.50 (d, 1H, $J = 4.7$ Hz, H_2), 8.54 (bs, 1H, H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.6 (CH_2, $C_{4'}$), 35.2 (CH_2, $C_{3'}$), 39.5 (CD_3, $J = 20.4$ Hz, N–CD_3) 56.9 (CH_2, $C_{5'}$), 68.7 (CH, $C_{2'}$), 123.4 (CH_{ar}, C_5), 134.5 (CD, $J = 24.1$ Hz, C_4), 138.6 (C^{IV}, C_3), 148.6 (CH_{ar}, C_6), 149.5 (CH_{ar}, C_2); MS (EI) *m/e* 166

(M^+, 11), 165 (10), 137 (15), 87 (100), 45 (17); ^2H NMR (61 MHz, CH_2Cl_2) δ 7.72 (1 ^2H, $^2H_4$), 2.09 (3 ^2H, C^2H_3).

[5-^{2}H-*N*-1′-C^{2}H$_3$]nicotine 5c

Following the protocol for the synthesis of **5d**, nicotine **5c** was obtained as oil (yield: 64%).

^1H NMR (300 MHz, $CDCl_3$) δ 1.66–2.07 (m, 3H, $H_{3'}+2H_{4'}$), 2.16–2.26 (m, 1H, $H_{3'}$), 2.26–2.36 (m, 1H, $H_{5'}$), 3.02–3.12 (m, 1H, $H_{2'}$), 3.20–3.30 (m, 1H, $H_{5'}$), 7.70 (bs, 1H, H_4), 8.50 (bs, 1H, H_2), 8.54 (bs, 1H, H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.6 (CH_2, $C_{4'}$), 35.2 (CH_2, $C_{3'}$), 39.5 (CD_3, $J=20.4$ Hz, N–CD_3), 56.9 (CH_2, $C_{5'}$), 68.8 (CH, $C_{2'}$), 123.2 (CD, $J=23.8$, C_5), 134.7 (CH_{ar}, C_4), 138.7 (C^{IV}, C_3), 148.6 (CH_{ar}, C_6), 149.5 (CH_{ar}, C_2); MS (EI) *m/e* 166 (M^+, 11), 165 (10), 137 (15), 87 (100), 45 (17); ^2H NMR (61 MHz, CH_2Cl_2) δ 7.27 (1 ^2H, $^2H_5$), 2.09 (3 ^2H, C^2H_3).

Conclusion

We have described the first total synthesis of four ^2H doubly labelled racemic nicotine regioisotopomers. These alkaloids were prepared in five steps with 7 to 24% overall yields starting from the corresponding commercially available halogeno nicotinates. The percentage of deuterium incorporation ranged from 90 to 92% on the pyridine moiety and 92 to 100% on the *N*-pyrrolidine substituent. The most pertinent doubly labelled nicotine was selected for further biological investigations.

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