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SYNTHESIS OF [4-$^2H_2$]-, (4R)[4-$^2H_1$]- AND (4S)[4-$^2H_1$]-4-(METHYLNITROSAMINO)-1-(3'-PYRIDYL)-1-BUTANONE, C-4 DEUTERIATED ISOTOPOMERS OF THE PROCARCINOGEN NNK.

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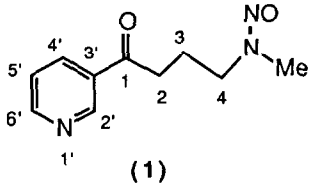
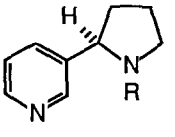
Abstract The synthesis of C-4 dideuteriated and both C-4 monodeuteriated enantiomers of NNK, the metabolic precursor to a variety of potential carcinogens, starting from (2S)-glutamic acid and nicotinic acid is described The route is suitable for the synthesis of NNK isotopomers labelled in each of the putative sites for metabolic activation

4-(Methylnitrosamino)-1-(3'-pyridyl)-1-butanone, NNK (1), N'-nitrosonornicotine, NNN (2) and related nitrosoamines are derived from the principal tobacco alkaloid, nicotine (3) Each of the compounds can be obtained through the nitrosation of nicotine [1] In recent years it has become apparent that many N-nitrosamines, including those present in many food products, are able to promote carcinogenesis [2]

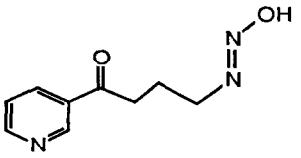


(1)



(2) R = NO, (3) R = Me

NNK is able to promote carcinogenesis in several assay systems[2-4] although the compound itself is not directly responsible and first needs to be activated [5] Ironically, activation can occur *in vivo* when the body attempts to detoxify and solubilise the compound For example, the diazohydroxides (4) and (5) could be formed from NNK through initial hydroxylation of the methyl group and the C-4 position, respectively [3,5] Each of these compounds is able to lose dinitrogen to give a highly reactive carbonium ion which could react with nearby nucleophiles, including those attached to proteins and DNA [6]



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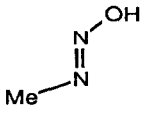


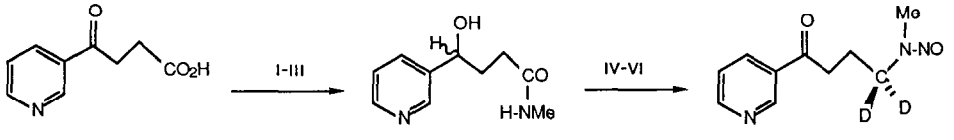
(5)

Although the current view is that the initial hydroxylation reactions are mediated by cytochrome P-450 enzymes,[5] the numbers and substrate specificities of the enzymes involved in these pathways have not been determined The situation is further confounded by the existence of *cis*- and *trans*- configurational isomers for unsymmetrical N,N-dialkyl N-nitrosamines, and the high energy barrier for their interconversion [7] Thus, it is not known which configurations of NNK are acted upon by the catabolic enzymes or which sites within each isomer are activated preferentially

In order to prepare substrates suitable for assaying each of these hydroxylase activities, it was necessary to be able to label NNK in each of the sites for potential hydroxylation separately and simultaneously Here we report on the synthesis of *trans*- C-4 dideuterated and C-4 chirally deuterated NNK isotopomers, starting from nicotinic acid and (2S)-glutamic acid, both of which can be prepared in a variety of labelled forms The rate of the conversion of the *trans*- isomer to the *cis*- isomer under the conditions prevailing during NNK metabolism is also assessed

DISCUSSION

Hecht and coworkers had reported a synthesis of [4-$^2H_2$]-NNK starting from 1-oxo-1-(3'-pyridyl)-butanoic acid (6)[8] which itself can be obtained in 38% yield from ethyl nicotinate [9] The acid was treated with oxalyl chloride and the resulting acid chloride was converted to the N-methyl butyramide through treatment with methylamine Borohydride reduction of the ketone gave the racemic alcohol (7) which was reduced to the dideuterated amino alcohol with hexadeuterio diborane Nitrosation of the product and, finally, chromium trioxide oxidation of the nitroso alcohol gave the desired dideuterated NNK in 3 4 % overall yield from ethyl nicotinate, Scheme 1

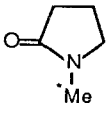


(6) (7)

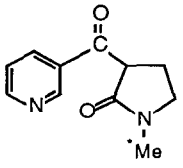
I , $(COCl)_2$, II , $MeNH_2$, III , BH_4^- , IV , B_2D_6 , V , HNO_2 , VI , CrO_3

Scheme 1

In order to prepare [*methyl*-$^2H_3$]-NNK, Hecht devised an alternative synthesis [8] Pyrrolidin-2-one was treated with sodium hydride and then with trideuteriomethyl mesylate The resulting N-trideuteriomethylpyrrolidinone (8) was Claisen condensed with ethyl nicotinate (9) in the presence of sodium hydride using the method of Eiden and coworkers [10] The product (10) was hydrolysed and decarboxylated by refluxing in concentrated HCl for 96 hours to give the dihydrochloride This was nitrosated to afford the deuteriated NNK (1, $^2H_3$-methyl) in 9 2% overall yield



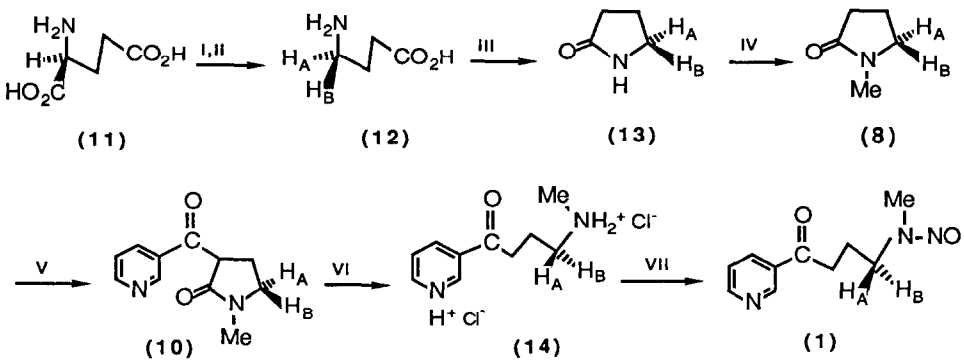
(8)



(10)

While the first synthetic route, Scheme 1, was unsuitable for our own purposes on many counts, it appeared that aspects of the second route might be useful For example, if we could prepare the pyrrolidin-2-ones chirally deuteriated at C-4 or dideuteriated at C-4 and incorporate these compounds into the remainder of Hecht's synthesis, the products would be the desired chirally deuteriated or dideuteriated NNKs In addition, the introduction of a labelled methyl group ([^{14}C]-, [$^2H_3$]-, or doubly labelled) into the same molecule would be facilitated and, furthermore, the use of labelled ethyl nicotinate (the free acid of which is commercially available in carbon-14 form) would provide a method for radio-labelling the two parts of the molecule which would become separated following hydroxylation [5] Access to these compounds offers an important advantage in facilitating hplc-based hydroxylase activity assays

It was envisaged that the chirally deuteriated pyrrolidin-2-ones would be accessible *via* the dehydration of chirally deuteriated γ-aminobutyric acids These can be prepared from the appropriately labelled (2S)-glutamic acids through an enzymic decarboxylation, in the appropriately labelled water, using *E coli* glutamate decarboxylase [11] While we were confident that the chirally deuteriated γ-aminobutyric acids could be converted to the corresponding pyrrolidinones without loss of label and without racemisation, we were concerned by the harsh conditions used by Hecht in the hydrolysis and decarboxylation of the keto amide (10) [8] Accordingly, the pyrrolidinone ring-closure and keto amide hydrolysis steps of the intended synthesis, Scheme 2, were examined using unlabelled materials



I , Aspartate Aminotransferase, $_{A}H_2O$, II , Glutamate Decarboxylase, $_{B}H_2O$, III , Alumina, Toluene, Reflux , IV , NaH, MeI , V , NaH, Ethyl Nicotinate , VI , 5M HCl, Reflux, 72 hours , VII , $NaNO_2$, pH 4 0, 16 hours

Scheme 2

Using a modification of the method of Blade'-Font,[12] γ-aminobutyric acid (12, H_A= H_B = H) and neutral alumina were heated under reflux in toluene in a Dean-Stark apparatus to give the essentially pure pyrrolidinone (13, H_A= H_B = H) in quantitative recovery A solution of the product in tetrahydrofuran was treated with sodium hydride and then methyl iodide to afford the N-methylpyrrolidinone (8, H_A= H_B = H) in 63% overall yield (lit[8] 49% for the single step) after purification by flash silica chromatography

The reported yield of the Claisen condensation of the methyl pyrrolidinone with ethyl nicotinate was 48% [8] This material contained upto 5% ethyl nicotinate, as judged by mass spectrometry and was not further purified Under optimised conditions we were able to obtain the Claisen condensation product (10, H_A= H_B = H) in 70% yield after purification by flash silica chromatography The product was ~90% pure as judged by ^{1}H- and ^{13}C-nmr and did not contain ethyl nicotinate as judged by mass spectrometry

The optimum conditions for the hydrolysis of the keto amide (10) were examined carefully since Hecht's reported conditions, refluxing 12M HCl, 96 hours, could potentially lead to racemisation or label loss in the compounds of interest to us [8] Marginally better yields of the dihydrochloride (~87%) than those reported were obtained under considerably milder conditions, refluxing 5M HCl, 72 hours However, all attempts to further reduce the harshness of the reaction conditions resulted in poor conversion yields The keto amide was converted to the *trans*-NNK with an overall yield from pyrrolidin-2-one of 16 5% Given that these modified conditions would be suitable for use in the synthesis of the chirally deuteriated isotopomers of NNK, the synthesis of the deuteriated pyrrolidinones was examined

(2S)-Glutamic acid (11) was decarboxylated in deuterium oxide using *E coli* glutamate decarboxylase (EC 4 1 1 15) [11] The product, (4R)-aminobutyric acid (12, H_A= H, H_B = ^{2}H), which was obtained in ~90% yield after ion exchange chromatography, was converted directly to the (5R)-pyrrolidinone (13, H_A= H, H_B = ^{2}H) in 74% overall yield The (5S)-enantiomer was prepared *via* an identical sequence starting from [2-$^2H_1$](2S)-glutamic acid and conducting the enzymic decarboxylation in protium oxide The deuteriated glutamic acid was prepared through aspartate aminotransferase (glutamic oxaloacetic acid transaminase, EC 2 6 1 1) catalysed exchange of the α-H atom of the unlabelled substrate (11) with the solvent, deuterium oxide A third, dideuteriated pyrrolidinone was prepared by decarboxylating (2S) [2-$^2H_1$]-glutamic acid in deuterium oxide and dehydrating the resulting [4-$^2H_2$]-4-aminobutyric acid (12, H_A= H_B = ^{2}H) on alumina in the usual way Each of the three deuteriated pyrrolidinones were then converted to the corresponding NNKs using the procedures described above, Scheme 2 All of the labelled compounds showed the expected nmr and mass spectral parameters and, evidently, no loss of label had occurred during the syntheses

The synthetic route outlined above gives predominantly the *trans*-NNK products Indeed, if the nitrosation reaction is worked-up rapidly, see experimental section, the *cis*-isomer is barely detectable Nevertheless, it was discovered that under simulated physiological conditions, aqueous buffer solution ~pH 7 at 37 °C, *trans*-NNK is converted to the *cis*- isomer at a significant rate Since the result of this observation, which appears not to have been noticed previously, has an important bearing on the delineation of the chemistry involved in the metabolism of NNK, the isomerisation reaction was examined more closely

The two C^{α} positions, the methyl group and the 4-methylene group are each well resolved in the ^{1}H- and ^{13}C-nmr spectra of the two isomers Note that the spectra were, in fact, assigned on the basis of DEPT and COSY experiments The ^{1}H-nmr spectrum of the *trans*- isomer shows signals at 3 06 and 4 26 ppm for the methyl and methylene groups respectively, whereas, the *cis*-isomer shows signals at 3 79 and 3 71 ppm The corresponding signals in the ^{13}C-nmr spectra occur at 31 4 and 52 9 ppm for the *trans*- isomer and at 38 9 and at 43 9 ppm for the *cis*- isomer

Using synthetic *trans*-NNK the isomerisation reaction was followed by ^{1}H-nmr spectroscopy at 37 °C in 98% deuteriated aqueous solution over the pL range 5 75-7 83 Under these conditions the *trans*-NNK reacted only to give the *cis*-isomer and an equilibrium mixture of the *cis*- and *trans*- isomers (1 4) was formed within 8 hours The first order rate constant for the isomerisation as calculated from these experiments was k_{isom} = 0 6 h^{-1} Therefore, the half-life for the *trans*- isomer, $t_{1/2}$ is 1 15 hours Under conditions where the *cis*- isomer is specifically removed, for example, through *cis*- specific hydroxylation, 90% of the *trans*- isomer could be consumed (*ie* ~3 half lives), without being acted upon directly, within a period of 4 hours This

result indicates that in determining which metabolic pathways operate during NNK catabolism, it is imperative to examine enzyme activities which operate on the minor (least stable) isomer

A cell-free preparation of bovine liver microsomes has been prepared recently in our laboratory The synthetic NNKs described here are metabolised by this preparation and, therefore, the compounds do not contain inhibitory impurities The purification of the enzymes present in this preparation will ultimately allow the kinetics and mechanism of the carcinogenic activation of NNK to be determined using the compounds described here

Experimental:

Melting point was determined using an electrothermal melting point apparatus and was uncorrected ^{1}H-nmr spectra at 270 MHz and ^{13}C-nmr spectra at 23 7 MHz were recorded on Jeol JNM-GX270 and Jeol FX90Q instruments Chloroform or sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-$^{2}H_4$ were used as standards for ^{1}H-nmr and chloroform or dioxane were used to reference ^{13}C-nmr Mass spectra were obtained using an AEI-MS30 spectrometer Microanalysis facilities were provided on a service basis by University College London, UK Specific rotations were determined on an Optical Activity Ltd AA-100 polarimeter using 10 cm path-length cells

2-Pyrrolidinone (13, $H_A = H_B = H$)· A mixture of 4-aminobutyric acid (12, $H_A = H_B = H$, 5 2 g, 50 mmol) and neutral alumina (15 g, 150 mmol) in toluene (250 ml) was heated under reflux using Dean - Stark apparatus After 10 h, the mixture was cooled and filtered Alumina was washed several times using a mixture of methanol and chloroform (1 1) The solvent was evaporated to give an oil Yield Quantitative δ_H ($CDCl_3$) 3 39 (2H, t, $J_{4,5}$ = 6 9 Hz, H-5), 2 29 (2H, m, H-3), 2 12 (2H, m, H-4), δ_C 179 2 (C-2), 42 2 (C-5), 30 0 (C-3), 20 6 (C-4) m/z (EI) 85 (M^+, 100), 42 (66), 41 (64), 30 (56)

1-Methyl-2-Pyrrolidinone (8, $H_A = H_B = H$). A suspension of sodium hydride (80% in oil, 0 45 g, 15 mmol) in dry tetrahydrofuran (20 ml) was cooled to 0^{o}C To this mixture, 2-pyrrolidinone (13, $H_A = H_B = H$, 0 42 g, 5 mmol) in the same solvent (5 ml) was added dropwise and the mixture was stirred for 20 min Methyl iodide (1 5 ml, 25 ml) was added slowly and the mixture was stirred overnight at room temperature The mixture was filtered through celite The celite bed was washed several times with tetrahydrofuran The filtrate and the washings were pooled together and evaporated to dryness The residue was partitioned between water and chloroform and the water layer was washed with chloroform (3 x 15 ml) Organic layers were pooled together, dried on

magnesium sulphate, filtered and dried *in vacuo* to give a brown oil The compound was purified by column chromatography on silica gel using a mixture of hexane dichloromethane (1 1) followed by dichloromethane as eluents Yield 0 31 g (63%) δ_H ($CDCl_3$) 3 35 (2H, t, $J_{5,4}$ = 7 1 Hz, H-5), 2 8 (3H, s, CH_3), 2 33 (2H, t, $J_{3,4}$ = 8 1 Hz, H-3), 1 98 (2H, quin, J_{gem} = 15 4 Hz, H-4) δ_C 49 1 (C-5), 30 3 (C-3), 29 1 (CH_3), 17 4 (C-4) m/z (EI) 99 (M^+, 100), 98 (70), 70 (14), 44 (80), 42 (57)

3-(3'-Pyridyl)-1-Methylpyrrolidin-2-one (10, H_A = H_B = H): A solution of compound 8, H_A = H_B = H (1 0 g, 10 mmol) in dry tetrahydrofuran (5 ml) was added dropwise to a suspension of sodium hydride (80% in oil, 0 9 g, 30 mmol) in the same solvent (40 ml) and the mixture was stirred for 15 min at room temperature A solution of ethyl nicotinate (3 g, 20 mmol) in dry tetrahydrofuran (5 ml) was added to it and the mixture was heated under reflux with efficient stirring After 24 h the mixture was cooled and carefully poured into ice-cold hydrochloric acid (50 ml, 4 N) After adjusting the pH of the solution to 4 (10 N sodium hydroxide solution), the aqueous solution was extracted with chloroform (6x15 ml) The combined extracts were dried *in vacuo* and redissolved in chloroform (50 ml) The solution was dried with magnesium sulphate and then concentrated *in vacuo* The residue was purified by column chromatography on silica gel using a mixture of hexane dichloromethane (1 1) followed by hexane dichloromethane (1 3) and then dichloromethane Appropriate fractions were pooled together and concentrated to give a yellow oil Yield 1 4 g (70%) δ_H ($CDCl_3$) 9 28 (1H, d, H-2'), 8 76 (1H, dd, $J_{5',6'}$ = 4 8 Hz, H-6'), 8 42 (1H, dt, $J_{4',5'}$ = 8 1 Hz, H-4'), 7 42 (1H, ddd, H-5'), 4 42 (1H, dd, H-3), 3 62-3 33 (2H, m, H-5), 2 85 (3H, s, CH_3), 2 75-2 63 and 2 31-2 17 (2H, m, H-4) δ_C 195 2 (pyridyl CO), 169 3 (C-2), 153 4 (C-6'), 150 7 (C-2'), 136 7 (C-4'), 131 5 (C-3'), 123 2 (C-5'), 50 9 (C-3), 47 9 (C-5), 29 8 (CH_3), 21 0 (C-4) m/z (EI) 204 (M^+, 23), 161 (64), 148 (12), 106 (100), 98 (63), 78 (75) The nmr spectra for this compound and its labelled isotopomers were assigned on the basis of a 2D COSY spectrum

4-(Methylamino)-1-(3'-pyridyl)-1-butanone dihydrochloride (14, H_A = H_B = H): A solution of compound 10, H_A = H_B = H (2 g, 10 mmol) in hydrochloric acid (5 N, 100 ml) was heated under reflux for 72 h The solution was cooled and evaporated to dryness The residue was redissolved in water, filtered and the filtrate was concentrated The title compound was recrystallised from methanol-ether mixture Yield 2 2 g (87%) δ_H (D_2O) 9 32 (1H, s, H-2'), 9 02 (1H, d, $J_{4',5'}$ = 8 3 Hz, H-4'), 8 98 (1H, d, $J_{5',6'}$ = 5 8 Hz, H-6'), 8 18 (1H, t, H-5'), 3 38 (2H, t, $J_{2,3}$ = 6 9 Hz, H-2), 3 17 (2H, t, $J_{3,4}$ = 7 8 Hz, H-4), 2 76 (3H, s, CH_3), 2 17 (2H, m, H-3) δ_C 197 6 (C-1), 146 4 (C-6'), 145 2 (C-4'), 142 4 (C-2'), 135 6 (C-3'), 128 8 (C-5'), 49 0 (C-4), 36 6 (C-2), 33 8 (CH_3), 20 1 (C-3) m/z (FAB+) 179 [$(M+H)^+$-2HCl, 100], 161 (14), 148 (18) The nmr spectra for this compound and its labelled isotopomers were assigned on the basis of a 2D COSY spectrum

4-(Methylnitrosamino)-1-(3'-pyridyl)-1-butanone (1, $H_A = H_B = H$): Compound 14, $H_A = H_B = H$ (2 5 g, 10 mmol) was dissolved in water (25 ml) and the solution was cooled to 0°C The pH of the solution was adjusted to 4 with 1N NaOH A solution of sodium nitrite (1 2 g, 17 5 mmol) in water (1 7 ml) was added dropwise and the reaction mixture was stirred at room temperature After 16 h, the aqueous solution was extracted with dichlororomethane (5x20 ml) The combined organic layers were washed successively with 4N NaOH (2x15 ml) and then water (3x15 ml) The solution was dried over magnesium sulphate and then concentrated *in vacuo* to give an oil The residue was recrystallised from dichloromethane ether to give the product Yield 0 9 g (43%) M p 71-73°C [13] Calc for $C_{10}H_{13}N_3O_2$ C 57 96, H 6 32, N 20 27 Found C 57 71, H 6 23, N 19 97 δ_H ($CDCl_3$) 9 14 (1H, s, H-2'), 8 79 (1H, d, H-6'), 8 2 (1H, d, H-4'), 7 42 (1H, dd, $J_{4',5'} = 8$ 1 Hz, $J_{5',6'} =$ 4 8 Hz, H-5'), 4 25 (t, $J_{3,4} = 6$ 7 Hz, H-4, *trans* isomer), 3 79 (s, CH_3, *cis* isomer), 3 71 (t, $J_{3,4} = 7$ 0 Hz, H-4, *cis* isomer), 3 09-3 04 (m, H-2 and CH_3, *trans* isomer), 2 94 (t, H-2, *cis* isomer), 2 22 (quin , H-3, *trans* isomer), 1 97 (quin , H-3, *cis* isomer) δ_C 197 4 (C-1), 153 7 (C-6'), 149 5 (C-2'), 135 2 (C-4'), 131 8 (C-3'), 123 7 (C-5'), 52 9 (C-4, *trans* isomer), 43 9 (C-4, *cis* isomer), 38 9 (CH_3, *cis* isomer), 35 9 (C-2, *cis* isomer), 35 2 (C-2, *trans* isomer), 31 4 (CH_3, *trans* isomer), 21 9 (C-3, *trans* isomer), 20 2 (C-3, *cis* isomer) m/z (EI) 177 ($[M - NO]^+$, 94), 159 (14), 148 (24), 146 (37), 118 (21), 106 (100), 78 (89) The nmr spectra for this compound and its labelled isotopomers were assigned on the basis of DEPT and COSY spectra

(5R)-[5-$^2H_1$]-2-Pyrrolidinone (13, $H_A = H$, $H_B = {}^2H$): (2S)-Glutamic acid (11), (1 48 g, 10 mmol) was suspended in deuterium oxide (25 ml) and the pH was adjusted to 5 with conc ammonia solution (in D_2O) Glutamate decarboxylase (150 units) and pyridoxal 5'-phosphate (5 mg) were added and the reaction was incubated at 37°C The pH was maintained at 5 by adding conc deuterium chloride solution every 15-20 minutes and the course of the reaction was monitored by tlc (solvent system isopropanol aq ammonia water 26 6 5) After 6 h the enzyme was denatured and the mixture was filtered The filtrate was concentrated *in vacuo* to give an oil The residue was dissolved in minimum amount of water and applied to a column of Amberlite IR45 (OH) (100 ml settled volume) The column was eluted with water Appropriate fractions were pooled and then concentrated *in vacuo* to give (4R)-[4-$^2H_1$]-4-aminobutyric acid (12, $H_A = H$, $H_B = {}^2H$) as a semi-solid It was converted to the title compound following the procedure reported above Yield 0 64g (74% on the basis of compound 11) δ_H ($CDCl_3$) 3 36 (1H, t, H-5), 2 27 (2H, t, H-3), 2 09 (2H, dd, H-4) δ_C 179 4 (C-2), 41 9 (t, $J_{CD} = 21$ 5 Hz, C-5), 30 0 (C-3), 20 5 (C-4) m/z (EI) 86 (M^+, 100), 43 (63), 42 (57), 31 (50)

1-Methyl-(5R)-[5-$^2H_1$]-2-Pyrrolidinone (8, $H_A = H$, $H_B = {}^2H$): Yield 1 4g (69%) δ_H ($CDCl_3$) 3 32 (1H, m, H-5), 2 79 (3H, s, CH_3), 2 32 (2H, t, H-3), 1 96 (2H, dd, H-4) δ_C 48 7 (t, J_{CD} = 22 2 Hz, C-5), 30 3 (C-3), 29 1(CH_3), 17 2 (C-4) m/z (EI) 100 (M^+, 100), 99 (52), 98 (30), 71

(15), 45 (82), 43 (51)

(5R)-[5-$^2H_1$]-3-(3'-Pyridyl)-1-Methylpyrrolidin-2-one (10, H_A = H, H_B = ^2H): Yield 1 6 g (78%) δ_H ($CDCl_3$) 9 27 (1H, d, H-2'), 8 75 (1H, dd, H-6'), 8 41 (1H, dt, H-4'), 7 41 (1H, dd, H-5'), 4 42 (1H, dd, H-3), 3 58-3 36 (1H, m, H-5), 2 84 (3H, s, CH_3), 2 73-2 63 and 2 28-2 17 (2H, m, H-4) δ_C 195 1 (pyridyl CO), 169 3 (C-2), 153 4 (C-6'), 150 7 (C-2'), 136 7 (C-4'), 131 4 (C-3'), 123 2 (C-5'), 50 9 (C-3), 47 5 (t, J_{CD} = 21 5 Hz, C-5), 29 8 (CH_3), 20 9 (C-4) m/z (EI) 205 (M^+, 24), 161 (65), 148 (7), 106 (100), 99 (59), 78 (73)

(4R)-[4-$^2H_1$]-4-(Methylamino)-1-(3'-pyridyl)-1-butanone dihydrochloride (14, H_A = H, H_B = ^2H) Yield 2 1 g (87%) δ_H (D_2O) 9 31 (1H, s, H-2'), 9 02-8 95 (2H, m, H-4' and H-6'), 8 15 (1H, dd, H-5'), 3 36 (2H, t, H-2), 3 11 (1H, t, H-4), 2 75 (3H, s, CH_3) 2 14 (2H, dd, H-3) δ_C 197 7 (C-1), 146 2 (C-6'), 145 4 (C-4'), 142 5 (C-2'), 135 6 (C-3'), 128 7 (C-5'), 48 6 (t, J_{CD} = 22 1 Hz, C-4), 36 6 (C-2), 33 7 (CH_3), 19 9 (C-3) m/z (FAB+) 180 [(M+H)$^+$-2HCl, 100], 162 (4), 149 (5)

(4R)-[4-$^2H_1$]-4-(Methylnitrosamino)-1-(3'-pyridyl)-1-butanone (1, H_A = H, H_B = ^2H): Yield 0 4 g (38%) δ_H ($CDCl_3$) 9 14 (1H,s, H-2'), 8 78 (1H, d, H-6'), 8 2 (1H, dd, H-4'), 7 42 (1H, dd, H-5'), 4 24 (m, H-4, *trans* isomer), 3 79 (s, CH_3, *cis* isomer), 3 69 (m, H-4, *cis* isomer), 3 09-3 03 (m, H-2 and CH_3, *trans* isomer), 2 95 (t, H-2, *cis* isomer), 2 22 (dd, H-3, *trans* isomer), 1 97 (dd, H-3, *cis* isomer) δ_C 197 4 (C-1), 153 5 (C-6'), 149 5 (C-2'), 135 2 (C-4'), 131 9 (C-3'), 123 7 (C-5'), 52 5 (t, J_{CD} = 21 5, C-4, *trans* isomer), 43 6 (C-4, *cis* isomer), 38 8 (CH_3, *cis* isomer), 35 8 (C-2, *cis* isomer), 35 1 (C-2, *trans* isomer), 31 2 (CH_3, *trans* isomer), 21 8 (C-3, *trans* isomer), 19 9 (C-3, *cis* isomer) m/z (EI) 178 ([M - NO]$^+$, 88), 160 (12), 149 (34), 147 (31), 119 (21), 106 (100), 78 (94)

(2S)-[2-$^2H_1$]-Glutamic acid hydrochloride (15): (2S)-Glutamic acid (11), (2 9 g, 20 mmol) was suspended in deuterium oxide (50 ml) and the pH was adjusted to 7 25 with conc ammonia solution (in D_2O) Glutamic oxaloacetic transaminase (200 units) and pyridoxal 5'-phosphate (5 mg) were added to this solution The mixture was incubated at 37°C and the reaction was monitored by ^{1}H-nmr After 72 h the enzyme was denatured and the mixture was filtered The filtrate was concentrated *in vacuo* The residue was dissolved in water (25 ml), concentrated and redissolved in water (10 ml) Concentrated hydrochloric acid solution was added dropwise until the title compound crystallised out yield 3 4 g (92%) $[\alpha]^{20}$= +27 1° (c 2 0 in 6 M HCl) [14] δ_H (D_2O) 3 79 (residual proton, t, H-2), 2 52 (2H, t, H-4), 2 12 (2H, m, H-3) δ_C 181 9 (C-1), 175 4 (C-5), 55 6 (m, C-2), 34 4 (C-4), 27 8 (C-3)

(5S)-[5-$^2H_1$]-2-Pyrrolidinone (13, H_A = ^2H, H_B = H): (2S)-[2-$^2H_1$]-Glutamic acid hydrochloride 15 (2 8 g, 15 mmol) was decarboxylated in water as reported above to give (4S)-[4-$^2H_1$]-4-aminobutyric acid (12, H_A = ^2H, H_B = H) It was then converted to the title compound following the procedure reported above Yield 0 98 g (76% on the basis of compound 15) δ_H ($CDCl_3$) 3 36 (1H, t, H-5), 2 27 (2H, t, H-3), 2 08 (2H, dd, H-4) δ_C 179 2 (C-2), 41 7 (t, J_{CD} = 21 5 Hz, C-5), 29 9 (C-3), 20 3 (C-4) m/z (EI) 86 (M^+, 100), 43 (68), 42 (63), 31 (57)

1-Methyl-(5S)-[5-$^2H_1$]-2-Pyrrolidinone (8, H_A = ^2H, H_B = H): Yield 1 1 g (67%) δ_H ($CDCl_3$) 3 34 (1H, m, H-5), 2 81 (3H, s, CH_3), 2 34 (2H, t, H-3), 1 98 (2H, dd, H-4) δ_C 48 9 (t, J_{CD} = 21 5 Hz, C-5), 30 5 (C-3), 29 3 (CH_3), 17 4 (C-4) m/z (EI) 100 (M^+, 100), 99 (55), 98 (34), 71 (15), 45 (85), 43 (55)

(5S)-[5-$^2H_1$]-3'-(3-Pyridyl)-1-Methylpyrrolidin-2'-one (10, H_A = ^2H, H_B = H): Yield 0 76 g (74%) δ_H ($CDCl_3$) 9 29 (1H, d, H-2'), 8 76 (1H, dd, H-6'), 8 41 (1H, dt, H-4'), 7 41 (1H, ddd, H-5'), 4 42 (1H, dd, H-3), 3 58-3 37 (1H, m, H-5), 2 84 (3H, s, CH_3), 2 74-2 64 and 2 29-2 15 (2H, m, H-4) δ_C 195 1 (pyridyl CO), 169 3 (C-2), 153 4 (C-6'), 150 7 (C-2'), 136 7 (C-4'), 131 5 (C-3'), 123 2 (C-5'), 50 9 (C-3), 47 5 (t, J_{CD} = 22 1 Hz, C-5), 29 8 (CH_3), 20 9 (C-4) m/z (EI) 205 (M^+, 20), 161 (58), 148 (7), 106 (100), 99 (56), 78 (74)

(4S)-[4-$^2H_1$]-4-(Methylamino)-1-(3'-pyridyl)-1-butanone dihydrochloride (14, H_A = ^2H, H_B = H): Yield 1 2 g (82%) δ_H (D_2O) 9 36 (1H, s, H-2'), 9 1 (1H, d, H-6'), 9 01 (1H, d, H-4'), 8 24 (1H, dd, H-5'), 3 42 (2H, t, H-2), 3 16 (1H, t, H-4), 2 77 (3H, s, CH_3) 2 16 (2H, dd, H-3) δ_C 146 2 (C-6'), 145 4 (C-4'), 142 5 (C-2'), 128 7 (C-5'), 48 7 (t, J_{CD} = 22 1 Hz, C-4), 36 6 (C-2), 33 7 (CH_3), 19 9 (C-3) m/z (FAB+) 180 [$(M+H)^+$-2HCl, 100], 162 (8), 149 (10)

(4S)-[4-$^2H_1$]-4-(Methylnitrosamino)-1-(3'-pyridyl)-1-butanone (1, H_A = ^2H, H_B = H). Yield 0 4 g (38%) δ_H ($CDCl_3$) 9 12 (1H, d, H-2'), 8 76 (1H, dd, H-6'), 8 19 (1H, dt, H-4'), 7 41 (1H, dd, H-5'), 4 23 (m, H-4, *trans* isomer), 3 78 (s, CH_3, *cis* isomer), 3 68 (m, H-4, *cis* isomer), 3 08-3 03 (m, H-2 and CH_3, *trans* isomer), 2 94 (t, H-2, *cis* isomer), 2 21 (dd, H-3, *trans* isomer), 1 96 (dd, H-3, *cis* isomer) δ_C 197 4 (C-1), 153 5 (C-6'), 149 2 (C-2'), 135 0 (C-4'), 131 8 (C-3'), 123 4 (C-5'), 52 4 (t, J_{CD} = 20 8 Hz, C-4, *trans* isomer), 38 6 (CH_3, *cis* isomer), 35 5 (C-2, *cis* isomer), 34 9 (C-2, *trans* isomer), 31 0 (CH_3, *trans* isomer), 21 6 (C-3, *trans* isomer), 19 7 (C-3, *cis* isomer) m/z (EI) 208 (M^+, 1), 178 ($[M - NO]^+$, 81), 160 (10), 149 (28), 147 (29), 119 (21), 106 (100), 78 (94)

[4,4-$^2H_2$]-2-Pyrrolidinone (13, H_A = H_B = ^2H): (2S)-Glutamic acid (11), (2 9 g, 20 mmol) in deuterium oxide (50 ml) was converted to (2S)-[2-$^2H_1$]-Glutamic acid following the procedure

reported above After 72 h the pH of the reaction mixture was readjusted to 5 with conc deuterium chloride solution (2S)-[2-$^2H_1$]-Glutamic acid was then decarboxylated and converted to the title compound as reported above Yield 1 6 g (92%, on the basis of compound 11) δ_H ($CDCl_3$) 3 34 (residual protons, H-5), 2 26 (2H, m, H-3), 2 06 (2H, t, H-4) δ_C 179 0 (C-2), 29 8 (C-3), 20 1 (C-4) m/z (EI) 87 (M^+, 100), 44 (78), 43 (72), 32 (69)

1-Methyl-[4,4-$^2H_2$]-2-Pyrrolidinone (8, $H_A = H_B = {}^2H$): Yield 1 28 g (73%) δ_H ($CDCl_3$) 3 32 (residual protons, ~8%, H-5), 2 79 (3H, s, CH_3), 2 32 (2H, t, H-3), 1 96 (2H, t, H-4) δ_C 174 4 (C-2), 30 1 (C-3), 28 9 (CH_3), 16 9 (C-4) m/z (EI) 101 (M^+, 100), 100 (32), 99 (54), 71 (16), 46 (86), 43 (62)

[5,5-$^2H_2$]-3-(3'-Pyridyl)-1-Methylpyrrolidin-2-one (10, $H_A = H_B = {}^2H$): Yield 2 0 g (81%) δ_H ($CDCl_3$) 9 28 (1H, d, H-2'), 8 76 (1H, dd, H-6'), 8 41 (1H, dt, H-4'), 7 41 (1H, ddd, H-5'), 4 42 (1H, dd, H-3), 3 58-3 33 (residual protons, m, H-5), 2 84 (3H, s, CH_3), 2 71-2 64 and 2 26-2 18 (2H, 2 x dd, H-4) δ_C 195 2 (pyridyl CO), 169 3 (C-2), 153 3 (C-6'), 150 6 (C-2'), 136 7 (C-4'), 131 5 (C-3'), 123 2 (C-5'), 50 9 (C-3), 29 8 (CH_3), 20 8 (C-4) m/z (EI) 206 (M^+, 23), 161 (57), 149 (12), 106 (100), 100 (50), 78 (68)

[4,4-$^2H_2$]-4-(Methylamino)-1-(3'-pyridyl)-1-butanone dihydrochloride (14, $H_A = H_B = {}^2H$)· Yield 2 1 g (83%) δ_H (D_2O) 9 34 (1H, s, H-2'), 9 04 (1H, m, H-6'), 8 99 (1H, d, H-4'), 8 2 (1H, dd, H-5'), 3 37 (2H, t, H-2), 2 76 (3H, s, CH_3) 2 14 (2H, t, H-3) δ_C 197 5 (C-1) 146 4 (C-6'), 145 2 (C-4'), 142 4 (C-2'), 135 5 (C-3'), 128 7 (C-5'), 36 6 (C-2), 33 7 (CH_3), 19 9 (C-3) m/z (FAB+) 181 [$(M+H)^+$-2HCl, 100], 163 (5), 150 (12)

[4,4-$^2H_2$]-4-(Methylnitrosamino)-1-(3'-pyridyl)-1-butanone (1, $H_A = H_B = {}^2H$): Yield 0 38 g (36%), 7 1 *trans cis* δ_H ($CDCl_3$) 9 08 (1H, d, H-2'), 8 72 (1H, dd, H-6'), 8 15 (1H, dt, H-4'), 7 37 (1H, dd, H-5'), 4 19 (m, residual protons, H-4, *trans* isomer), 3 74 (s, CH_3, *cis* isomer), 3 05-2 99 (m, H-2 and CH_3, *trans* isomer), 2 9 (t, H-2, *cis* isomer), 2 17 (t, H-3, *trans* isomer), 1 91 (t, H-3, *cis* isomer) δ_C 197 4 (C-1), 153 4 (C-6'), 149 2 (C-2'), 135 0 (C-4'), 131 8 (C-3'), 123 4 (C-5'), 38 6 (CH_3, *cis* isomer), 35 6 (C-2, *cis* isomer), 35 0 (C-2, *trans* isomer), 31 1 (CH_3, *trans* isomer), 21 5 (C-3, *trans* isomer), 19 7 (C-3, *cis* isomer) m/z (EI) 209 (M^+, 1), 179 ([M - NO]$^+$, 66), 161 (23), 150 (25), 148 (20), 119 (20), 106 (100), 78 (84)

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