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Research Article

Efficient and practical syntheses of a stable labelled form of the non-narcotic analgesic, bicifadine and its metabolites

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Abstract: An efficient and practical synthesis of deuterium-labelled bicifadine, together with 3 deuterium-labelled bicifadine metabolites, was developed. The levels of deuterium incorporation are high, and the products were used as internal standards in bioanalytical method development and clinical bioanalysis. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: bicifadine; (±)-1-(4-methylphenyl)-3-azabiocyclo[3.1.0]hexane; deuterium; DOV 220,075

Introduction

Bicifadine [(±)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]-hexane hydrochloride; DOV 220,075 HCl, Figure 1] is a non-narcotic analgesic under development for the treatment of acute and chronic pain. It is effective in the treatment of acute dental pain[1,2] and postoperative bunionectomy pain.[3] It is also active in models of neuropathic pain, with no narcotic-like withdrawal symptoms.[4] Bicifadine's primary pharmacological action is to enhance and prolong the actions of norepinephrine and serotonin by inhibiting the transport proteins that terminate the physiological actions of these two biogenic amines. The analgesic efficacy of orally administered 75 and 150 mg bicifadine hydrochloride was compared to 650 mg aspirin and placebo in a double-blind, single-dose study.[5] Significant analgesic activity was reported with 650 mg aspirin and 150 mg bicifadine compared to placebo, and side effects were reported to be minor. In additional studies in dental surgery patients, bicifadine produced analgesia comparable to the narcotic, codeine and the narcotic-like agent tramadol, respectively.[5]

To support our on-going clinical studies, there was a need for the synthesis of deuterium-labelled bicifadine, along with the syntheses of a number of deuterium-labelled bicifadine metabolites (Figure 2).

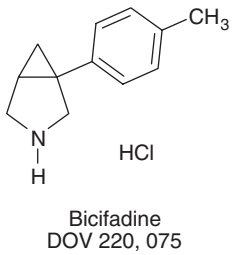

Figure 1 Bicifadine HCl.

Results and discussion

The original reported synthesis of bicifadine was an inefficient, seven-step procedure.[1] Some improvements of the synthesis of 1-aryl-3-aza-bicyclo[3.1.0]hexanes have recently been reported in patent literature.[6] Here we report a simple and effective synthesis of deuterium-labelled bicifadine, illustrated in Scheme 1. Using the same approach, we also developed novel and practical routes to synthesize three deuterium-labelled bicifadine metabolites.

Reaction of 4-tolylacetonitrile (**1**) with [$^2H_5$]-epichlorohydrin (**2**)[7] using the literature disclosed base, sodium amide,[8] gave inconsistent results. After screening several bases, we discovered that sodium hexamethyldisilazide was a much better base for this reaction, with the best results achieved when 2 equivalents of sodium hexamethyldisilazide were added at low temperature in a stepwise fashion. Compound **3** was generated as a

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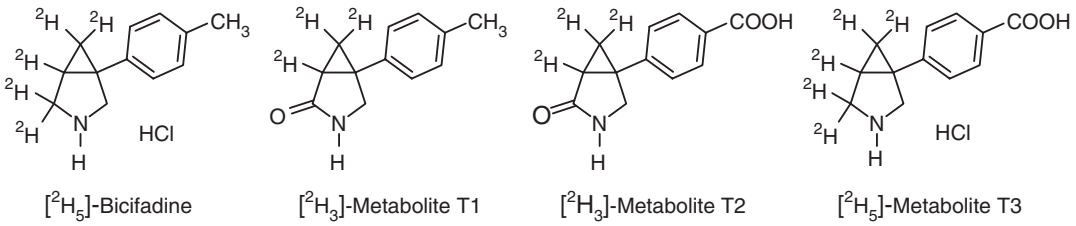
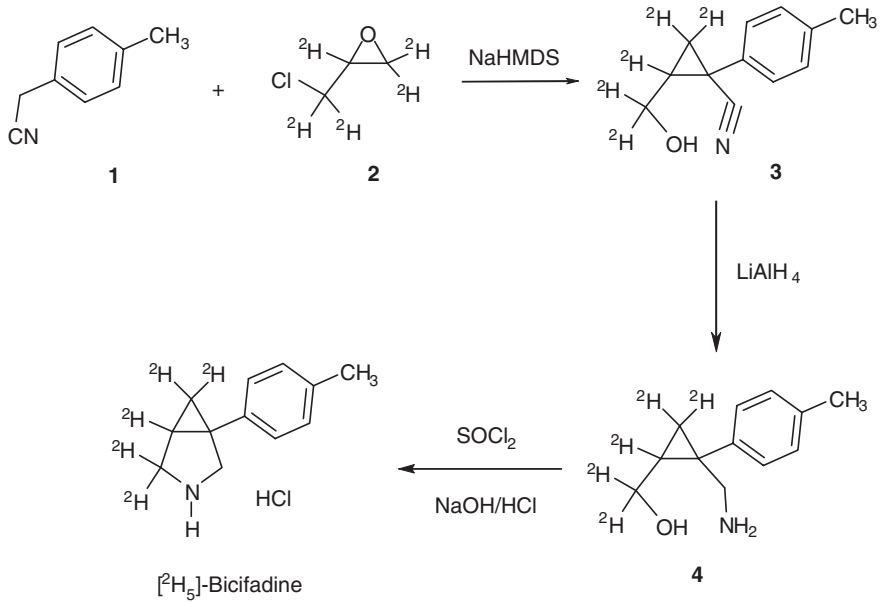


Figure 2 Deuterium-labelled bicifadine and its metabolites.



Scheme 1 Synthesis of [$^2H_5$]-bicifadine.

mixture of *cis* and *trans* isomers with a ratio of about 4:1. These isomers were carried forward into the next steps without separation because only the *cis* isomer can cyclize to form the final target compound. Treatment of [$^2H_5$][2-(aminomethyl)-2-(4-methylphenyl)cyclopropyl]methanol (**4**) with thionyl chloride, followed by sodium hydroxide and finally hydrochloric acid yielded [$^2H_5$]-bicifadine in good yield.

A similar synthetic strategy was applied to the synthesis of bicifadine [$^2H_3$]-labelled metabolite T1 starting with intermediate **4** (Scheme 2).

The key step was the oxidative cyclization of the *t*-BOC-protected amino alcohol (**5**). Interestingly, pyridinium dichromate in dichloromethane, which had worked for the non deuterium-labelled analogue, did not proceed well when reacting with **5**. A multicomponent mixture was observed. A possible explanation is that the carbon–deuterium bond is stronger than the carbon–hydrogen bond and a stronger oxidative condition is needed. In general, PDC is a stronger oxidant in DMF than in DCM,[9] and literature has reported that oxidation of deuterated primary alcohols could be achieved with pyridinium dichromate in dimethylformamide.[10] When this was applied to compound (**5**), oxidation and cyclization occurred to yield the lactam (**6**), which was readily transformed into T1 by TFA deprotection.

The successful synthesis of labelled bicifadine and metabolite T1 might have provided good intermediates for a quick oxidation of the tolyl methyl group to the corresponding benzoic acids, producing metabolites T2 and T3. However, the oxidation step proved not to be trivial. The lactam **6** was subjected to oxidation with potassium permanganate in aqueous pyridine (Scheme 3). This reaction was extremely time- and temperature- dependent. If the reaction was performed for too long and/or the reaction temperature too high (i.e. >80°C), decomposition of the product occurred, and it is hypothesized that product (**8**) is formed, possibly via a hydrolytic ring cleavage reaction.

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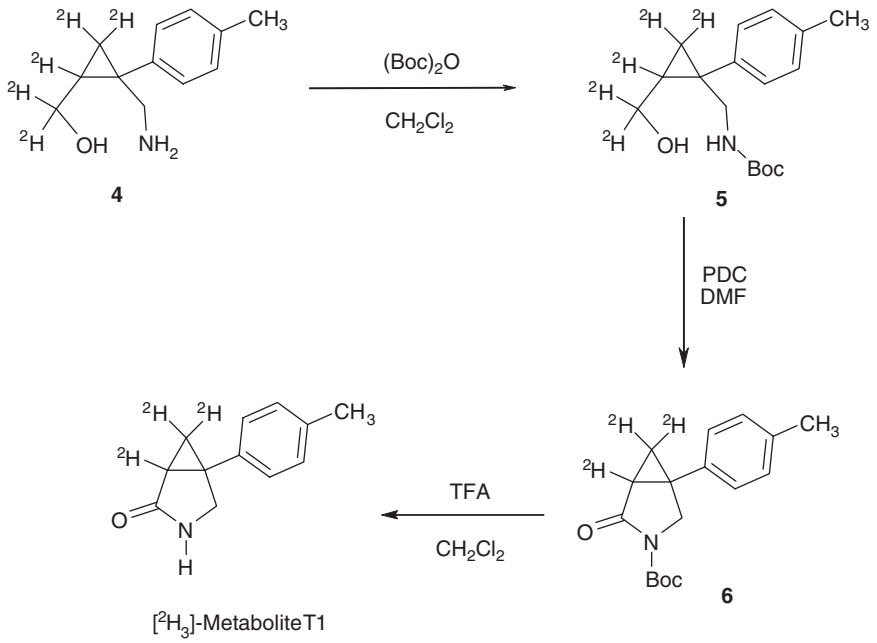
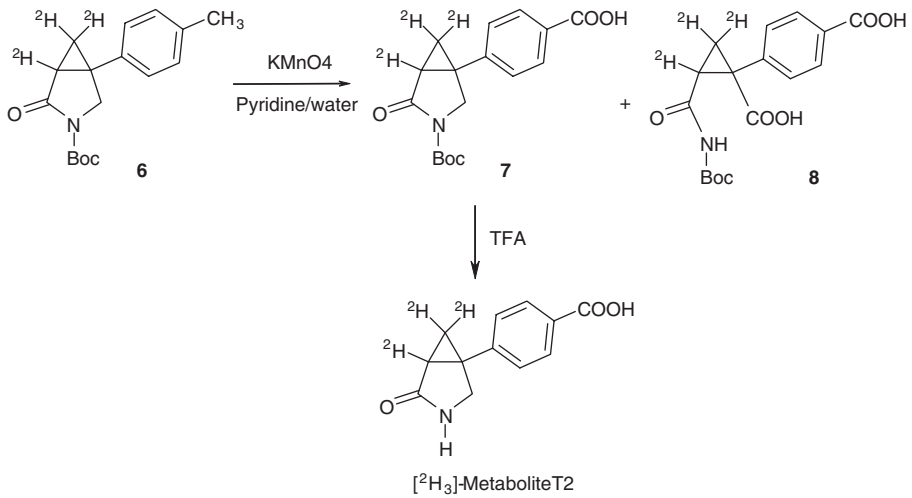


Scheme 2 Synthesis of [$^2H_3$]-bicifadine metabolite T1.



Scheme 3 Synthesis of [$^2H_3$]-bicifadine metabolite T2.

With careful control of temperature and time, the acid intermediate (**7**) could be isolated. Although a considerable amount of the starting material remained, it could be removed and recycled using an acid–base extraction technique. The intermediate (**7**) needed to be purified by preparative HPLC in order to remove the hydrolysed material (**8**). TFA deprotection afforded metabolite T2.

The oxidation technique was attempted for the preparation of metabolite T3 from [$^2H_5$]-bicifadine. This resulted in a complex mixture in which over-oxidation and ring hydrolysis occurred. We have explored and developed a novel route for T3 as illustrated in Scheme 4. Using the methods shown in Schemes 1 and 2, deuterium-labelled 1-(4-bromophenyl)-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**13**) was prepared in slightly lower yield than its methyl analogue. Treatment of **13** with zinc cyanide in a microwave resulted in the desired cyano compound (**14**), which was readily hydrolysed with sodium

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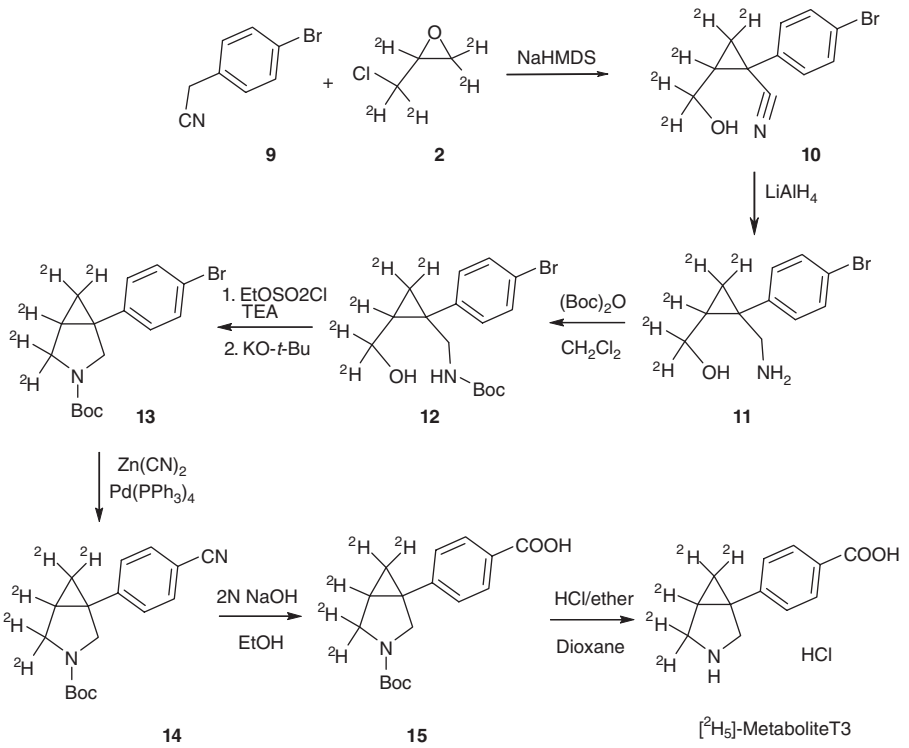

Scheme 4 Synthesis of [$^2H_5$]-bicifadine metabolite T3.

hydroxide and subsequently deprotected with hydrochloric acid/diethyl ether to give metabolite T3 as the hydrochloride salt.

Experimental

Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel plates and visualized with potassium permanganate or ultra-violet light. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded in $CDCl_3$. Unless otherwise noted, peak positions are give in parts per million downfield from tetramethylsilane and *J*-values are given in hertz. Organic solutions were dried over magnesium sulphate and evaporated using a rotary evaporator. Purifications that were performed by HPLC used a C18 column, eluant 40% methanol in water up to 100% methanol (0.1% formic acid).

([$^2H_5$])2-(Hydroxymethyl)-1-(4-methylphenyl)cyclopropanecarbonitrile (3)

To a stirred solution of 4-methylbenzonitrile (**1**) (2.01, 15.37 mmol) in dry tetrahydrofuran (40 ml), cooled at −20°C under nitrogen was added sodium hexamethyldisilazide (15.5 ml, 15.37 mmol of 1 M solution in tetrahydrofuran) drop wise. The resultant solution was stirred at −15°C for 30 minutes. The mixture was re-cooled to −20°C and a solution of [$^2H_5$]-epichlorohydrin (**2**) (1.5 g, 0, 15.37 mmol) in tetrahydrofuran (10 ml) was added dropwise. The reaction was stirred at a temperature between −9 and −15°C for 30 minutes. Finally the reaction mixture was re-cooled to −20°C and sodium hexamethyldisilazide (15.5 ml, 15.37 mmol of 1 M solution in tetrahydrofuran) was added dropwise. The reaction mixture was stirred for 1.5 hours allowing the mixture to warm to about 10°C. The resulting solution was re-cooled to 0°C and quenched with water (15 ml). The reaction mixture was further diluted with water (20 ml) and extracted with ethyl acetate (3 × 30 ml). The combined organic extract was washed with hydrochloric acid (2 M, 30 ml) dried, filtered and evaporated to a red oil (3.42 g). The residue was purified on a 50 g silica cartridge eluting with 20% ethyl acetate in hexane and then 30% ethyl acetate in hexane to afford [$^2H_5$]2-(hydroxymethyl)-1-(4-methylphenyl)cyclopropanecarbonitrile (**3**) as a clear oil (1.56 g, 53%) as a mixture of *cis* and *trans* isomers (~4:1) ^{1}H NMR *cis* isomer at δ 2.35 (s, 3H), 7.15 (m, 4H). Appropriate peaks were observed for the *trans* isomer at δ, 2.38 and 7.25.

([$^2H_5$])(2-(Aminomethyl)-2-(4-methylphenyl)cyclopropyl)methanol (4)

To a stirred solution of lithium aluminium hydride (32.4 ml, 0.032 mol of 1.0 M solution in ether) in dry

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diethyl ether (190 ml) at 0°C under nitrogen was added a solution of [$^2H_5$]2-(hydroxymethyl)-1-(4-methylphenyl)cyclopropanecarbonitrile (**3**) (1.56 g, 8.11 mmol) in diethyl ether (10 ml) dropwise over 10 minutes causing an immediate precipitation. The resultant suspension was allowed to warm to room temperature and stirred overnight. The reaction was re-cooled to 0°C and quenched first with ethyl acetate (20 ml) and then cautiously with water (50 ml); the mixture was diluted with further ethyl acetate (50 ml) and filtered through hyflo; the aqueous layer was extracted with ethyl acetate (30 ml). The combined organic extracts were dried, filtered and evaporated to give [$^2H_5$][2-(aminomethyl)-2-(4-methylphenyl)cyclopropyl]methanol (**4**) as a colourless oil (1.55 g, quantitative), as a mixture of *cis* and *trans* isomers, which was used without further purification, crude ^{1}H NMR δ 2.35 (s, 3H), 2.6 (d, 1H $J = 12$), 3.4 (d, 1H $J = 12$), 7.1 (d, 2H $J = 8$), 7.3 (d, 2H $J = 8$). Appropriate peaks were observed for the *trans* isomer at δ2.35, 2.45, 3.05 and 7.2.

([$^2H_5$])1-(4-Methylphenyl)-3-azabicyclo(3.1.0)hexane hydrochloride (([$^2H_5$])-Bicifadine)

To a stirred solution of [$^2H_5$][2-(aminomethyl)-2-(4-methylphenyl)cyclopropyl]methanol (**4**) (0.55 g, 2.80 mmol) in anhydrous dichloroethane (7 ml) was added thionyl chloride (0.27 ml, 3.70 mmol) dropwise. The reaction was exothermic and gave a mild effervescence. The mixture was stirred at room temperature for 3.75 hours. The mixture was quenched with water (15 ml) and dichloromethane (20 ml) was added. The organic extract was extracted with water (2×30 ml) and the combined aqueous extracts were basified with 10 M sodium hydroxide and extracted with dichloromethane (3×60 ml), the combined organic extracts were dried, filtered and evaporated to an oily, yellow solid. This residue was dissolved in methanol (10 ml) and excess ethereal hydrochloric acid was added and then the mixture concentrated. The resulting cream solid was dissolved in methanol and solid impurities were removed by filtration and the filtrate was evaporated to a cream solid. This solid was repeatedly triturated with acetonitrile to yield [$^2H_5$]1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane hydrochloride (**[$^2H_5$]-Bicifadine**) as a white solid (340 mg, 57%) ^{1}H NMR DMSO δ 2.26 (m, 3H), 3.40 (d, 1H), 3.63 (d, 1H), 7.13 (m, 4H). Deuterium purity 97.8% $^2H_5$, 2.0% $^2H_4$, 0.2% $^2H_3$.

([$^2H_5$])(2-(Hydroxymethyl)-1-(4-methylphenyl)cyclopropylmethyl)carbamic acid *tert*-butyl ester (5)

To a stirred solution of [$^2H_5$][2-(aminomethyl)-2-(4-methylphenyl)cyclopropyl]methanol (**4**) (7.89 mmol) in dichloromethane (50 ml) was added di-*tert*-butylcarbonate (1.90 g, 8.69 mmol) in one portion. The reaction flask was fitted with a bubbler and a slow evolution of gas was observed. The reaction mixture was stirred at room temperature for 3 hours. This was washed with water (30 ml) and the aqueous layer was extracted with dichloromethane (30 ml); the combined organic extracts were washed with saturated sodium bicarbonate (30 ml), water (30 ml), dried, filtered and evaporated to give a yellow oil (3.0 g). This oil was purified on a 50g silica cartridge eluting with hexane to 30% ethyl acetate in hexane to give [$^2H_5$][2-(hydroxymethyl)-1-(4-methylphenyl)cyclopropylmethyl]carbamic acid *tert*-butyl ester (**5**) as a clear oil (1.48 g, 64%) ^{1}H NMR δ 1.4 (s, 9H), 2.35 (s, 3H), 3.57 (m, 2H), 4.8 (bs, 1H), 7.1 (d, 2H $J = 8$) 7.2 (d, 2H $J = 8$).

([$^2H_3$])1-(4-Methylphenyl)-4-oxo-3-azabicyclo(3.1.0)-hexane-3-carboxylic acid *tert*-butyl ester (6)

To a stirred solution of [$^2H_5$][2-(hydroxymethyl)-1-(4-methylphenyl)cyclopropylmethyl]carbamic acid *tert*-butyl ester (**5**) (0.6 g, 2.0 mmol) in dimethylformamide (20 ml) was added pyridinium dichromate (2.63 g, 7.0 mmol). The resultant mixture was stirred overnight at room temperature. The reaction mixture was diluted with water (20 ml) and 2 M hydrochloric acid (5 ml). The mixture was extracted with diethyl ether (2×30 ml). The organic extracts were washed with water (4×20 ml), dried, filtered and evaporated to a clear gum (530 mg). The material was purified on a 20 g silica cartridge eluting with hexane to 10% ethyl acetate in hexane to a give [$^2H_3$]1-(4-methylphenyl)-4-oxo-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**6**) as a colourless oil (288 mg, 51%), which solidified on standing, ^{1}H NMR δ 1.52 (s, 9H), 2.35 (s, 3H), 3.95 (d, 1H $J = 11$) 4.0 (d, 1H $J = 11$), 7.15 (m, 4H).

([$^2H_3$])5-(4-Methylphenyl)-3-azabicyclo(3.1.0)hexane-2-one (([$^2H_3$])-Metabolite T1)

To a stirred solution of [$^2H_3$]1-(4-methylphenyl)-4-oxo-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**6**) (0.25 g, 0.84 mmol) in dichloromethane (10 ml) was added trifluoroacetic acid (0.648 ml, 8.4 mmol). The reaction mixture was stirred at room temperature for 2 hours during which time gas evolution was observed. The reaction was evaporated to give a yellow gum (140 mg), which was purified by prep hplc to yield [$^2H_3$]5-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane-2-one (**[$^2H_3$]-Metabolite T1**) as a cream solid (58 mg, 36%) ^{1}H NMR δ 2.36 (s, 3H), 3.69 (dd, 2H), 5.33 (bs, 1H), 7.17 (m, 4H), deuterium purity, 98.3% $^2H_3$, 1.0% $^2H_2$, 0.1% ^2H, 0.6% unlabelled.

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($^2H_3$)1-(4-Carboxyphenyl)-4-oxo-3-azabicyclo (3.1.0)hexane-3-carboxylic acid *tert*-butyl ester (7)

A solution of [$^2H_3$]1-(4-methylphenyl)-4-oxo-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**6**) (0.68 g, 2.33 mmol) in pyridine (40 ml)) was treated with potassium permanganate (2.21 g, 13.98 mmol) and water (10 ml); the resultant mixture was heated at 80°C internal temperature for 5.5 hours. After cooling, the reaction mixture was diluted with water (100 ml) and acidified with 2 M hydrochloric acid and extracted with ethyl acetate (3 × 50 ml); the combined organic extracts were washed with 2 M hydrochloric acid (50 ml), water (50 ml), dried, filtered and evaporated to colourless oil (0.31 g). The oil was dissolved in dimethylsulphoxide (2 ml) and purified by preparative HPLC to afford [$^2H_3$]1-(4-carboxyphenyl)-4-oxo-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**7**) as a white solid (51 mg, 7%) ^{1}H NMR δ 1.55 (s, 9H), 4.05 (d, 1H $J = 11$), 4.15 (d, 1H $J = 11$), 7.45 (d, 2H $J = 8$), 8.15 (d, 2H $J = 8$).

($^2H_3$)4-(4-Oxo-3-azabicyclo(3.1.0)hex-1-yl)benzoic acid (($^2H_3$)-Metabolite T2)

To a stirred solution of [$^2H_3$]1-(4-carboxyphenyl)-4-oxo-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**7**) (51 mg, 0.159 mmol) in dichloromethane (2 ml) was added trifluoroacetic acid (0.50 ml). An immediate gas evolution commenced and the mixture was stirred at room temperature for 2 hours. The excess solvent was evaporated to give a clear gum (41 mg). This gum was triturated with diethyl ether to yield [$^2H_3$]4-(4-oxo-3-azabicyclo[3.1.0]hex-1-yl)benzoic acid (**[$^2H_3$]-DOV Metabolite T2**) a white solid (35 mg, quantitative) ^{1}H NMR DMSO δ 3.64 (s, 2H), 7.35 (d, 2H), 7.4 (bs, 1H), 7.89 (d, 2H), 12.9 (bs, 1H), deuterium purity, 99% $^2H_3$, 1.0% $^2H_2$.

($^2H_5$)1-(4-Bromophenyl)-2-(hydroxymethyl)cyclopropanecarbonitrile (10)

To a stirred solution of 4-bromobenzylcyanide (**9**) (4.0 g 0.0204 mol) in dry tetrahydrofuran (120 ml), cooled at −20°C under nitrogen was added sodium hexamethyldisilazide (20.6 ml, 0.0206 mol of 1 M solution in tetrahydrofuran) dropwise. The resultant solution was stirred at −15°C for 30 minutes. The mixture was re-cooled to −20°C and a solution of [$^2H_5$]-epichlorohydrin (**2**) (2.00 g, 0,0204 mol) in tetrahydrofuran (20 ml) was added dropwise. The reaction was stirred at a temperature between −9 and −15°C for 30 minutes. Finally the reaction mixture was re-cooled to −20°C and sodium hexamethyldisilazide (20.6 ml, 0.0206 mol of 1 M solution in tetrahydrofuran) was added drop wise. The reaction mixture was stirred for 1.5 hours allowing the mixture to warm to about 10°C. The resulting solution was re-cooled to 0°C and quenched with water (30 ml). The reaction mixture was further diluted with water (50 ml) and extracted with ethyl acetate (3 × 75 ml). The combined organic extract was washed with hydrochloric acid (2 M, 100 ml) dried, filtered and evaporated to a gummy solid (5.03 g). The residue was purified on a 100 g silica cartridge eluting with 15% ethyl acetate in hexane and then 30% ethyl acetate in hexane to afford [$^2H_5$]1-(4-bromo-phenyl)-2-hydroxymethyl-cyclopropanecarbo-nitrile (**10**) as a clear gum (2.20 g 42%) as a mixture of *cis* and *trans* isomers (~3:1) ^{1}H NMR *cis* isomer δ 7.2 (d, H $J = 9$), 7.5 (d, H $J = 9$) other aromatic peaks were observed for the *trans* isomer and some impurities. The compound was not purified further.

($^2H_5$)(2-(Aminomethyl)-2-(4-bromophenyl)cyclopropyl)methanol (11)

To a stirred solution of lithium aluminium hydride (34 ml, 0.034 mol of 1.0 M solution in diethyl ether) in dry diethyl ether (175 ml) at 0°C under nitrogen was added a solution of [$^2H_5$]1-(4-bromo-phenyl)-2-hydroxymethyl-cyclopropanecarbonitrile (**10**) (2.15 g, 8.53 mmol) in diethyl ether (25 ml) dropwise over 25 minutes, causing immediate precipitation. The resultant suspension was allowed to warm to room temperature and was stirred at room temperature overnight. The reaction was re-cooled to 0°C and quenched first with ethyl acetate (10 ml) and then cautiously with water (50 ml). Once the quench was complete, the mixture was filtered through hyflo; the aqueous layer was extracted with ethyl acetate (2 × 50 ml). The combined organic extract was dried, filtered and evaporated to give [$^2H_5$][2-(aminomethyl)-2-(4-bromophenyl)cyclopropyl]methanol (**11**) as a clear oil (2.1 g, 94%) as a mixture of *cis* and *trans* isomers, which was used without further purification. ^{1}H NMR *cis* isomer δ 2.6 (d, 1H $J = 12$), 3.45 (d, 1H $J = 12$) 7.3 (d, H $J = 8$), 7.45 (d, H $J = 8$).

($^2H_5$)(1-(4-Bromophenyl)-2-(hydroxymethyl)cyclopropylmethyl)carbamic acid *tert*-butyl ester (12)

To a stirred solution of [$^2H_5$][2-(aminomethyl)-2-(4-bromophenyl)cyclopropyl]methanol (**11**) (2.05 g, 7.85 mmol) in dichloromethane (80 ml) was added di-*tert*-butylcarbonate (2.05 g, 9.38 mmol) in one portion, the reaction flask was fitted with a bubbler and a slow evolution of gas was observed. The reaction mixture was stirred at room temperature for 2 hours. This was

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washed with water (50 ml) and the aqueous layer was extracted with dichloromethane (30 ml); the combined organic extracts were washed with saturated sodium bicarbonate (50 ml), water (50 ml), dried, filtered and evaporated to give a yellow gum (3.2 g). This gum was purified on a 50 g silica cartridge eluting with hexane to 20% ethyl acetate in hexane to give [$^2H_5$][1-(4-bromophenyl)-2-(hydroxymethyl)cyclopropylmethyl]carbamic acid *tert*-butyl ester (**12**) as a clear gum (1.6 g, 56%) ^{1}H NMR δ 1.4 (s, 9H), 3.3 (m, 1H), 3.5 (m, 1H), 4.8 (bs, 1H), 7.2 (d, 2H $J = 8$) 7.4 (d, 2H $J = 8$).

($^2H_5$)1-(4-Bromophenyl)-3-azabicyclo(3.1.0)hexane-3-carboxylic acid *tert*-butyl ester (13)

To a stirred solution of [$^2H_5$][1-(4-bromophenyl)-2-(hydroxymethyl)cyclopropylmethyl]carbamic acid *tert*-butyl ester (**12**) (1.6 g, 4.43 mmol) in dichloromethane (40 ml) at 0°C was added ethane sulphonyl chloride (0.82 ml, 1.12 g, 8.86 mmol) and triethyl amine (0.9 g, 1.24 ml, 8.86 mmol). The mixture was stirred at room temperature for 1 hour after which the reaction was quenched with water (30 ml); the layers were separated and the aqueous phase was extracted with dichloromethane (30 ml). The combined organic extracts were washed with saturated sodium bicarbonate solution (3 0ml), dried, filtered and evaporated to yield an oil (2.5 g). This was dissolved in dry tetrahydrofuran (40 ml) and cooled to 0°C with stirring, to the resultant solution was added potassium-*tert*-butoxide (0.744 g, 6.64 mmol) causing a deep red colouration. The resultant mixture was stirred at 0°C for 1 hour and was then quenched with water (30 ml) and extracted with ethyl acetate (2 × 50 ml). The combined organic extract was dried, filtered and evaporated to a red oil (1.4 g). This was purified on a 20 g silica cartridge eluting with hexane to 10% ethyl acetate in hexane to afford [$^2H_5$]1-(4-bromophenyl)-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**13**) as a colourless oil (0.77 g, 51%) ^{1}H NMR δ 1.5 (s, 9H), 3.55 (m, 1H), 3.95 (m, 1H), 7.0 (m, 2H), 7.45 (d, 2H $J = 8$).

($^2H_5$)1-(4-Cyanophenyl)-3-azabicyclo(3.1.0)hexane-3-carboxylic acid *tert*-butyl ester (14)

A solution of [$^2H_5$]1-(4-bromophenyl)-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**13**) (234 mg, 0.68 mmol), zinc cyanide (79 mg, 0.68 mmol) and palladium tetrakis triphenylphosphine (78 mg, 0.068 mmol) in dimethylformamide (4 ml) in a sealed microwave vial was subjected to microwaves. The mixture was heated to 150°C over 1.5 minutes and then held at 150°C for 1.5 minutes. This was repeated on an equivalent scale a further 4 times. After cooling the 5 reaction mixtures were combined and diluted with water (40 ml) and extracted with ethyl acetate (2 × 50 ml). The organic extract was washed with water (4 × 50 ml), dried, filtered and evaporated to a brown gum (750 mg). The material was purified on a 20 g silica cartridge eluting with hexane to 20% ethyl acetate in hexane to afford [$^2H_5$]1-(4-cyanophenyl)-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**14**) as a clear gum (460 mg, 47%) ^{1}H NMR δ 1.45 (s, 9H), 3.6 (m, 2H) 4.0 (m, 2H), 7.2 (m, 2H), 7.6 (d, 2H $J = 8$).

($^2H_5$)1-(4-Carboxyphenyl)-3-azabicyclo(3.1.0)hexane-3-carboxylic acid *tert*-butyl ester (15)

To a stirred solution of [$^2H_5$]1-(4-cyanophenyl)-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**14**) (460 mg, 1.59 mmol) in absolute ethanol (50 ml) was added 2 M-sodium hydroxide solution (10 ml). The mixture was heated at 90°C overnight. After cooling, the solvent was evaporated, the residue was dissolved in water (10 ml) and extracted with ethyl acetate (10 ml); the organic extract was extracted with 2 M sodium hydroxide solution (2 × 10 ml). The combined aqueous extracts were acidified to pH 1 with 2 M hydrochloric acid and extracted with ethyl acetate (2 × 30 ml); the combined organic extracts were dried, filtered and evaporated to a yield [$^2H_5$]1-(4-carboxyphenyl)-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**15**) as a white solid (460 mg, 94%) ^{1}H NMR δ 1.5 (s, 9H), 3.65 (t, 1H $J = 10$), 4.0 (m, 1H), 7.25 (m, 2H), 8.05 (d, 2H $J = 8$).

($^2H_5$)4-(3-Azabicyclo(3.1.0)hex-1-yl)benzoic acid hydrochloride (($^2H_5$)-Metabolite T3)

To a stirred solution of [$^2H_5$]1-(4-carboxyphenyl)-3-azabicyclo[3.1.0]hexane-3-carboxylic acid *tert*-butyl ester (**15**) (460 mg, 1.5 mmol) in dioxane (10 ml) under nitrogen was added an anhydrous solution of hydrochloric acid (15 ml of 2 M solution in diethyl ether). Stirring was continued at room temperature for 2.5 hours during which time a precipitate had formed. The solvent was evaporated and the residue was triturated with ethyl acetate to afford [$^2H_5$]4-(3-azabicyclo[3.1.0]-hex-1-yl)benzoic acid hydrochloride (**[$^2H_5$]-Metabolite T3**) as a cream solid (230 mg, 63%) ^{1}H NMR DMSO δ 3.54 (d, 1H), 3.73 (d, 1H), 7.36 (d, 2H), 7.89 (d, 2H), 9.47 (bs, 2H), 12.83 (bs, 1H) deuterium purity, 98% $^2H_5$, 1.8% $^2H_4$, 0.2% unlabelled.

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