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Efficient syntheses of ^{13}C- and ^{14}C-labelled 5-benzyl and 5-indolylmethyl *L*-hydantoins

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Robust and straightforward methods are described for the first syntheses of highly pure ^{13}C- and ^{14}C-labelled *L*-5-benzylhydantoin (*L*-BH) and *L*-5-indolylmethylhydantoin (*L*-IMH) by cyclizing the amino acids *L*-phenylalanine and *L*-tryptophan, respectively, with potassium cyanate. [3-^{13}C]-*L*-phenylalanine was used to prepare [6-^{13}C]-*L*-BH and [indole-2-^{13}C]-*L*-tryptophan was used to prepare [indole-2-^{13}C]-*L*-IMH, which we required for solid-state NMR experiments with a hydantoin transport protein. The successful incorporation and integrity of the ^{13}C labels was confirmed by solution-state NMR spectroscopy. [^{14}C]Potassium cyanate was used to prepare [2-^{14}C]-*L*-BH and [2-^{14}C]-*L*-IMH, which we required for transport assays with the protein.

Keywords: hydantoins; 5-substituted; benzylhydantoin; indolylmethylhydantoin; ^{13}C-labelled; ^{14}C-labelled; transport proteins; solid-state NMR

Introduction

5-Substituted hydantoins are important precursors to optically pure α-amino acids for use in the food and pharmaceutical industries, principally *via* their enzyme-catalysed hydrolyses.[1–6] The hydantoin moiety is also found in natural products[7–9] and forms the basis of some pharmacologically active compounds including 5-benzylhydantoin anticonvulsants such as phenytoin,[10–12] inhibitors of the NMDA receptor,[13] and modulators of the androgen receptor.[14] As part of our studies on the structure–function relationships of hydantoin transporters in pathogenic bacteria,[15–19] we required a number of ^{13}C- and ^{14}C-labelled 5-monosubstituted hydantoins for solid-state NMR experiments and for transport assays, respectively, which are not commercially available. We have already reported the synthesis of ^{14}C-labelled *DL*-allantoin,[20] but we then required optically pure *L*-5-benzylhydantoin (*L*-BH) **1** and *L*-5-indolylmethylhydantoin (*L*-IMH) **2** (Figure 1), which are thought to be substrates for the Mhp1 transporter,[17–19] labelled separately with both ^{13}C and ^{14}C. Here, we describe our approach to the syntheses of ^{13}C/^{14}C-labelled *L*-BH and *L*-IMH by cyclizing the amino acids *L*-phenylalanine and *L*-tryptophan, respectively, with potassium cyanate.

Experimental

General

Chemicals and NMR solvents were from Sigma-Aldrich (unless stated otherwise), reaction solvents were of analytical grade and all used without further purification. Centrifugation was performed using an Eppendorf 5804 R bench-top centrifuge. Melting points were measured using a STUART Melting Point Apparatus. NMR spectra were recorded on a Bruker Avance 300 spectrometer at frequencies of 300.13 MHz for ^{1}H and 75.48 MHz for ^{13}C and on a Bruker Avance 500 spectrometer at frequencies of 500.23 MHz for ^{1}H and 125.80 MHz for ^{13}C; chemical shifts (δ) are given in ppm relative to the internal standard TMS. High-resolution mass spectra were obtained using a Waters GCT Premier instrument. Liquid scintillation analysis was performed on a Packard Tri-Carb 2100TR instrument and using an emulsifier-safe scintillation cocktail from Perkin-Elmer.

L-5-benzylhydantoin (unlabelled)

A mixture of *L*-phenylalanine (1.0 g, 6.05 mmol) and potassium cyanate (1.0 g, 12.33 mmol) in water (50 ml) was heated (50°C) and rotated on a rotary evaporator until all the phenylalanine had dissolved. The solution was then reduced under vacuum to ~10 ml. Dilute HCl (1 M) was added dropwise with stirring until the precipitate remained in solution (final pH ~5). The precipitate was collected by filtration to give *N*-carbamoyl-*L*-phenylalanine (634 mg, 3.05 mmol, 50%) as colourless crystals [Mp 192°C (lit. 188–190°C[21], 193°C[22]). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.45 (s, 1 H, OH), 7.31–7.17 (m, 5 H, Ar–H), 6.16 (d, 1 H, J = 8.3 Hz, NH), 5.63 (s, 2 H, NH_2), 4.32 (m, 1 H, J = 5.2 Hz and 10.8 Hz, CHCO), 2.92 (m, 2 H, J = 5.2 Hz, 7.8 Hz, and 13.9 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 174.3 (COOH), 158.5 ($CONH_2$), 137.9 (Ar–C), 129.6 (Ar–C), 128.5 (Ar–C), 126.7 (Ar–C), 54.1 (CH_2CH), 37.9 (CH_2). MS (ES$^+$/TOF) *m/z* 209.33 (MH^+, 100%), 231.34 (MNa^+, 35%), 247.30 (MK^+, 71%), 120.34 (63%), 166.36 (58%). Anal. calcd for $C_{10}H_{12}N_2O_3$: C, 57.69; H, 5.81; N, 13.45. Found: C, 56.45; H, 5.55; N, 13.45]. 500 mg (2.40 mmol) of the crystals were suspended in 2 M HCl (10 ml) and heated under

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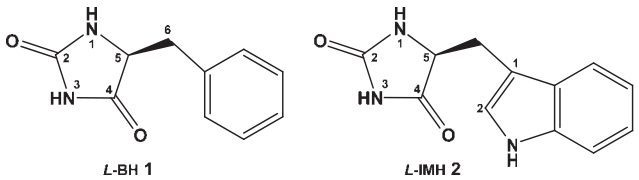


Figure 1. Structures of *L*-5-benzylhydantoin (*L*-BH) 1 and *L*-5-indolylmethylhydantoin (*L*-IMH) 2.

reflux for 2 h. The reaction mixture was allowed to cool to room temperature and the resultant precipitate was collected by filtration, washed with water, air-dried under vacuum on the filter and then dried under vacuum in a dessicator over P_2O_5 to give *L*-BH (357 mg, 1.88 mmol, 31% from *L*-phenylalanine, 78% from *N*-carbamoyl-*L*-phenylalanine) as colourless plates. Mp 181°C (lit. 181–183°C[25,24]). ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 10.43$ (s, 1 H, H-3), 7.92 (s, 1 H, H-1), 7.31–7.19 (m, 5 H, Ar-H), 4.34 (t, 1 H, $J_{H5,H6} = 5.1$ Hz, H-5), 2.94 (d, 2 H, $J_{H6,H5} = 5.1$ Hz, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 175.5$ (C-2), 157.5 (C-4), 136.0 (C-7), 130.1 (C-9), 128.4 (C-8), 127.0 (C-10), 58.7 (C-5), 36.8 (C-6). HRMS (ESI$^+$/TOF): *m/z* calcd for $C_{10}H_{10}N_2O_2+H^+ = 191.0815$; found: 191.0823 and calcd for $C_{10}H_{10}N_2O_2+Na^+ = 213.0634$; found: 213.0638. Anal. calcd for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.00; H, 5.20; N, 14.45.

[6-^{13}C]-*L*-5-benzylhydantoin

A mixture of [3-^{13}C]-*L*-phenylalanine (Cambridge Isotope Laboratories) (250 mg, 1.51 mmol) and potassium cyanate (245 mg, 3.02 mmol) in water (12.5 ml) was heated (50°C) and rotated on a rotary evaporator until all of the phenylalanine had dissolved. The solution was then reduced under vacuum to ~3 ml. Dilute HCl (1 M) was added dropwise with stirring until the precipitate remained in solution (final pH ~5). The precipitate was collected by filtration to give [3-^{13}C]-*N*-carbamoyl-*L*-phenylalanine (151 mg, 0.73 mmol, 48%) as colourless crystals. The crystals were suspended in 2 M HCl (2.5 ml) and heated under reflux for 2 h. The reaction mixture was allowed to cool to room temperature and the resultant precipitate was collected by filtration, washed with water, air-dried under vacuum on the filter and then dried under vacuum in a dessicator over P_2O_5 to give [6-^{13}C]-*L*-BH (84 mg, 0.44 mmol, 29% from *L*-[3-^{13}C]phenylalanine, 60% from *N*-carbamoyl-*L*-phenylalanine) as colourless plates. ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 10.41$ (s, 1 H, H-3), 7.90 (s, 1 H, H-1), 7.30–7.17 (m, 5 H, Ar–H), 4.32 (m, 1 H, $J_{H5,H6} = 5.1$ Hz, H-5), 2.94 (m, 2 H, $J_{H6,H5} = 5.1$ Hz, and $J_{H6,C6} = 129$ Hz, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 175.5$ (C-2), 157.5 (C-4), 136.0 (d, $J_{C7,C6} = 44.3$ Hz, C-7), 130.0 (C-9), 128.4 (d, $J_{C8,C6} = 3.8$ Hz, C-8), 127.0 (C-10), 58.7 (d, $J_{C5,C6} = 34.7$ Hz, C-5), 36.8 (^{13}C-enriched, C-6). MS (ES$^+$/TOF) *m/z* 191.8 (MH$^+$, 100%). HRMS (ESI$^+$/TOF): *m/z* calcd for $C_9^{13}CH_{10}N_2O_2+Na^+ = 214.0668$; found: 214.0669.

[2-^{14}C]-*L*-5-benzylhydantoin

The method was as above for [6-^{13}C]-*L*-BH except that 500 μCi [^{14}C]potassium cyanate (specific activity 40 mCi/mmol; American Radiolabelled Chemicals) was included with the original reaction mixture and unlabelled *L*-phenylalanine was used. [2-^{14}C]-*L*-BH (78 mg, 0.41 mmol) was obtained as colourless fine needles with a chemical yield of 27% from *L*-phenylalanine. 2.0 mg of the crystals gave ^{14}C counts (Packard Tri-Carb 2100TR) with an average of 6 036 855 dpm, which is equivalent to 100.61 kBq or 2.7193 μCi; therefore, 78 mg of the crystals has a total of 106.1 μCi and a ^{14}C specific activity of 258.6 μCi/mmol. The [^{14}C]potassium cyanate used in the reaction had 500 μCi; hence, the radiochemical yield was 106.1 μCi/500 μCi × 100 = 21%.

L-5-indolylmethylhydantoin (unlabelled)

A mixture of *L*-tryptophan (204.2 mg, 1.0 mmol) and potassium cyanate (97.3 mg, 1.2 mmol) in water (5 ml) was stirred at 70°C for 30 min (in a 25-ml round-bottomed flask). The solution was allowed to cool to room temperature and concentrated HCl (12 M, 1.04 ml) (final concentration = 2 M) was added followed by stirring at room temperature for 30 min; the resultant white precipitate is *N*-carbamoyl-*L*-tryptophan. A concentration of 2 M HCl (5 ml) was added, then the suspension was transferred to a 50-ml Falcon tube; the precipitate was collected by centrifugation (5000 rpm, 10 min, 4°C) and then washed twice with 2 M HCl (20 ml) with centrifugation after each wash. The precipitate was resuspended in 2 M HCl (20 ml), transferred to a 50-ml round-bottomed flask and stirred at 100°C for 30 min (formation of *L*-IMH). The mixture was transferred to a 50-ml Falcon tube and incubated on ice overnight to completely crystallize *L*-IMH. The crystals were collected by centrifugation, the supernatant was removed, and ice-cold water (20 ml) was added. The pH was adjusted to 7.0 by the addition of NaOH (2 M at first then 0.5 M) to dissolve any residual *N*-carbamoyl-*L*-tryptophan (Be very careful! *L*-IMH will be damaged at pH 9 and above). The crystals were collected by centrifugation, the supernatant was removed, and the crystals were washed with ice-cold water (20 ml). The crystals were collected by centrifugation, the supernatant was removed, and water (50 ml) was added. The suspension was transferred to a 100-ml round-bottomed flask and heated at 100°C until the crystals had dissolved, followed by hot filtration. The filtrate was incubated on ice overnight and the resultant crystals were collected by filtration (on a glass-frit filter with no vacuum), washed with ice-cold water (2 × 10 ml), air-dried under vacuum on the filter and then dried under vacuum in a dessicator over P_2O_5 to give *L*-IMH (112 mg, 0.49 mmol, 49% from *L*-tryptophan) as colourless fine needles. Mp ~250°C (lit. 244, 248–251°C[24]). ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 10.91$ (s, 1 H, N3-H), 10.36 (s, 1 H, N1-H), 7.91 (s, 1 H, indole-NH), 7.56 (d, 1 H, $J = 8.0$ Hz, indole-H), 7.33 (d, 1 H, $J = 8.0$ Hz, indole-H), 7.14 (d, 1 H, $J = 3.0$ Hz, indole-H2), 7.07−6.96 (m, 2 H, 2 × indole-H), 4.32 (t, 1 H, $J = 4.7$ Hz, H-5), 3.08 (d, 2 H, $J = 4.7$ Hz, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 176.1$ (C-2), 157.8 (C-4), 136.2 (indole-C), 127.8 (indole-C), 124.5 (indole-C2), 121.2 (indole-C), 118.9 (indole-C), 118.7 (indole-C), 111.6 (indole-C), 108.3 (indole-C1), 58.7 (C-5), 26.9 (CH_2). HRMS (ES$^-$/TOF): *m/z* calcd for $C_{12}H_{11}N_3O_2$-H = 228.0779; found: 228.0787. Anal. calcd for $C_{12}H_{11}N_3O_2$: C, 63.15; H, 4.42; N, 18.41. Found: C, 62.15; H, 4.85; N, 18.50.

[Indole-2-^{13}C]-*L*-5-indolylmethylhydantoin

The method was as above for unlabelled *L*-IMH except that 205.2 mg (1.0 mmol) of [indole-2-^{13}C]-*L*-tryptophan (Cambridge Isotope Laboratories) was used in the reaction mixture. [Indole-2-^{13}C]-*L*-IMH (96 mg, 0.42 mmol, 42%) was obtained as colourless fine needles. ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 10.91$ (s, 1 H, N3-H), 10.36 (s, 1 H, N1-H), 7.91 (s, 1 H, indole-NH), 7.56 (d, 1 H, $J = 8.0$ Hz, indole-H), 7.33 (d, 1 H, $J = 8.0$ Hz, indole-H), 7.14 (dd, 1 H, $J = 3.0$ Hz and 181 Hz, indole-H2), 7.07−6.96 (m, 2 H, 2 × indole-H), 4.32 (t, 1 H, $J = 4.7$ Hz, H-5), 3.08 (dd, 2 H,

$J = 4.7$ Hz and < 1 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 176.1$ (C-2), 157.8 (C-4), 136.2 (d, $J = 4.5$ Hz, indole-C), 127.8 (d, $J = 3.1$ Hz, indole-C), 124.5 (^{13}C-enriched, indole-C2), 121.2 (indole-C), 118.9 (d, $J = 4.6$ Hz, indole-C), 118.7 (indole-C), 111.6 (d, $J = 2.5$ Hz, indole-C), 108.3 (d, $J = 70.1$ Hz, indole-C1), 58.7 (C-5), 26.9 (d, $J = 4.5$ Hz, CH_2). MS (ES^+/TOF) *m/z* 231.38 (MH^+, 100%). HRMS (ESI^+/TOF): *m/z* calcd for $C^{13}_{11}CH_{11}N_3O_2 + H^+ = 231.0958$; found: 231.0960 and calcd for $C^{13}_{11}CH_{11}N_3O_2 + Na^+ = 253.0777$; found: 253.0782.

[2-^{14}C]-*L*-5-indolylmethylhydantoin

The method was as above for unlabelled *L*-IMH except that 500 μCi [^{14}C]potassium cyanate (specific activity 40 mCi/mmol; American Radiolabelled Chemicals) was included with the original reaction mixture (added in 1 ml of water out of a total volume of 5 ml). [2-^{14}C]-*L*-IMH (102 mg, 0.45 mmol) was obtained as colourless fine needles with a chemical yield of 45%. 2.0 mg of the crystals gave ^{14}C counts (Packard Tri-Carb 2100TR) with an average of 7 521 069 dpm, which is equivalent to 125.35 kBq or 3.3879 μCi; therefore, 102 mg of the crystals has a total of 172.8 μCi and a ^{14}C specific activity of 386.6 μCi/mmol. The [^{14}C]potassium cyanate used in the reaction had 500 μCi; hence, the radiochemical yield was 172.8 μCi/500 μCi × 100 = 35%.

Results and discussion

General strategy

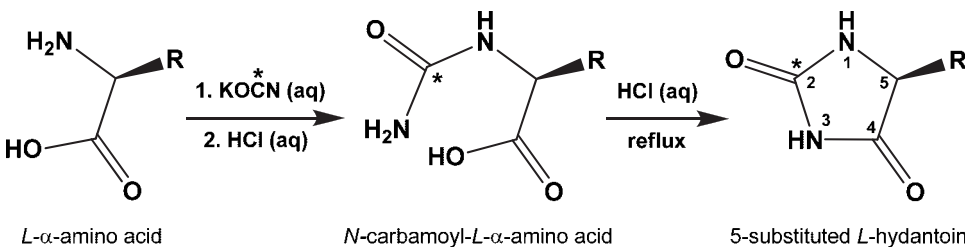
Our chosen method for synthesizing *L*-BH and *L*-IMH was to react the appropriate *L*-α-amino acid with potassium cyanate and hydrochloric acid, an approach first described by Urech in 1873[25] and variations on the procedure have been described by others since then, which notably includes much work by Dakin.[21,23,26–28] The α-amino acid reacts with potassium cyanate to form an *N*-carbamoyl-α-amino acid (or β-substituted α-uramidopropionic acid),[29,30], which is often isolated in crystalline form; this is cyclized under acid conditions to form the hydantoin ring, where C-5 corresponds to the α-carbon of the starting amino acid and C-5 substituents correspond to the side chain of the amino acid[29] (Scheme 1). A principal reason for choosing this method is that it retains optical activity,[30,31] as demonstrated by Dakin;[28] *L*-BH and *L*-IMH are therefore produced from *L*-phenylalanine and *L*-tryptophan, respectively. This was an important consideration for preparing the ^{14}C-compounds as it avoids separating enantiomers of the radiolabelled hydantoin products. Other important considerations in choosing the synthetic route were the availability and costs of ^{13}C/^{14}C-labelled starting materials or reagents, avoidance of technically demanding procedures (due to limited facilities for radiolabelled work), as few steps as possible or introduction of the ^{13}C/^{14}C label at the final step (to maximize labelling yield and to minimize handling of radiolabelled material), and ideally a robust method that produces the final hydantoins as crystalline solids with > 99% purity from relatively small-scale (~200 mg or less) reactions and avoiding chromatography. Our approach was to first synthesize unlabelled hydantoins to develop the method and handling procedures and to assess the identity and purity of the products before preparing the ^{13}C/^{14}C-labelled versions; this is described in more detail below and separately for *L*-BH and *L*-IMH.

L-5-Benzylhydantoin

In our synthesis of *L*-BH, we first isolated the *N*-carbamoyl-*L*-amino acid using a method similar to that described by Dakin,[21] but with some modifications. Equal quantities of *L*-phenylalanine and potassium cyanate in aqueous solution were simply heated and then the solution was acidified to ~pH 5 to give crystals of *N*-carbamoyl-*L*-phenylalanine (α-uramido-β-phenylpropionic acid) in 50% yield, which were verified by melting point, NMR, mass spectrometry, and elemental analyses. Reflux of *N*-carbamoyl-*L*-phenylalanine in hydrochloric acid followed by cooling afforded crystals of *L*-BH in 31% yield from *L*-phenylalanine and in 78% yield from *N*-carbamoyl-*L*-phenylalanine. The straightforward method and high purity of the unlabelled product allowed us to proceed with labelled versions of *L*-BH.

We required a ^{13}C-labelled version of *L*-BH for solid-state NMR experiments to detect the binding of *L*-BH to the Mhp1 hydantoin transport protein[17–19] expressed in bacterial inner membranes, as we have successfully carried out with the substrates for other transport systems.[32–36] C-6 was the target position for a ^{13}C label in *L*-BH as its ^{13}C NMR chemical shift at 36.8 ppm has the least overlap with signals coming from lipids and proteins in ^{13}C solid-state NMR spectra of bacterial inner membranes.[32–36] Starting from [3-^{13}C]-*L*-phenylalanine, the synthesis was performed on a 250-mg scale to give 84 mg of [6-^{13}C]-*L*-5-benzylhydantoin in a yield of 29% from the amino acid. The ^{13}C NMR spectrum of [6-^{13}C]-*L*-BH (Figure 2(A)) shows exclusive ^{13}C-enrichment at C-6 along with characteristic ^{13}C–^{13}C splitting of the signals for the adjacent positions C-5 and C-7 with coupling constants of 34.7 Hz and 44.3 Hz, respectively. In the ^{1}H NMR spectrum, the H-6 methylene signal at 2.94 ppm shows a ^{1}H-^{13}C coupling constant of 129 Hz (Figure 2(C) and (D)).

A radiolabelled version of *L*-BH was required for our assays to measure the substrate uptake into bacterial cells *via* hydantoin transporters.[17] The reagent common to the syntheses of both *L*-BH and *L*-IMH, potassium cyanate, was used to introduce a ^{14}C-label at C-2 in the hydantoin ring (Scheme 1). The synthesis



L-α-amino acid — *N*-carbamoyl-*L*-α-amino acid — 5-substituted *L*-hydantoin

Scheme 1. Synthesis of 5-substituted *L*-hydantoins from *L*-α-amino acids. For *L*-phenylalanine, *N*-carbamoyl-*L*-phenylalanine and *L*-5-benzylhydantoin $R = CH_2Ph$; for *L*-tryptophan, *N*-carbamoyl-*L*-tryptophan and *L*-5-indolylmethylhydantoin $R = CH_2$-indole. The asterisk shows the position of the ^{14}C label when [^{14}C]potassium cyanate was used in the reaction.

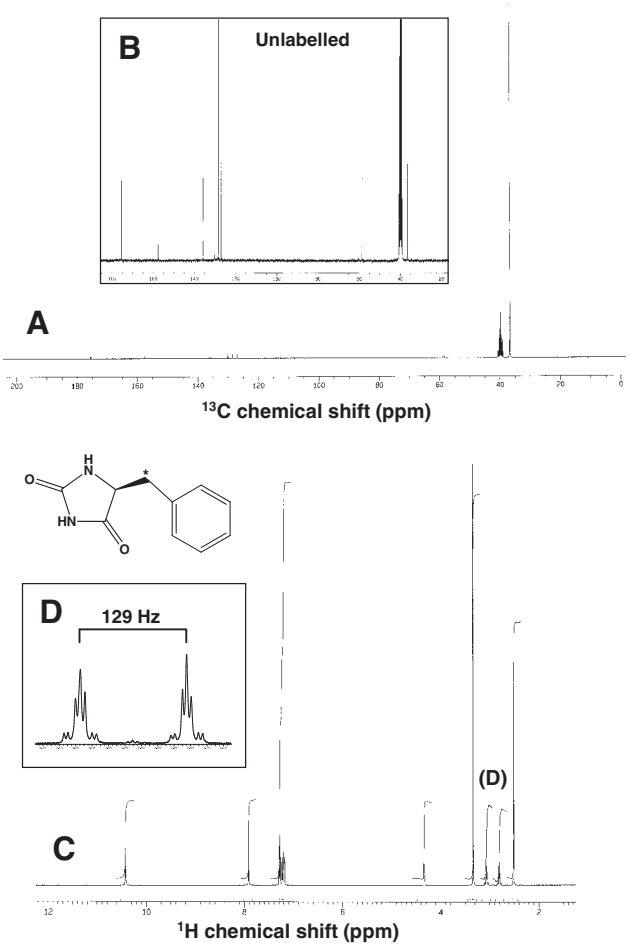


Figure 2. NMR analysis of [6-^{13}C]-*L*-5-benzylhydantoin. (A and B) ^{13}C NMR spectra of [6-^{13}C]-*L*-5-benzylhydantoin and unlabelled *L*-5-benzylhydantoin, respectively, in DMSO-d_6 obtained at 75.48 MHz. (C and D) ^{1}H NMR spectrum of [6-^{13}C]-*L*-5-benzylhydantoin in DMSO-d_6 obtained at 500.23 MHz; D shows an expansion of the H-6 methylene signal and its coupling with the ^{13}C label at C-6. The asterisk shows the position of the ^{13}C label on the structure of *L*-BH.

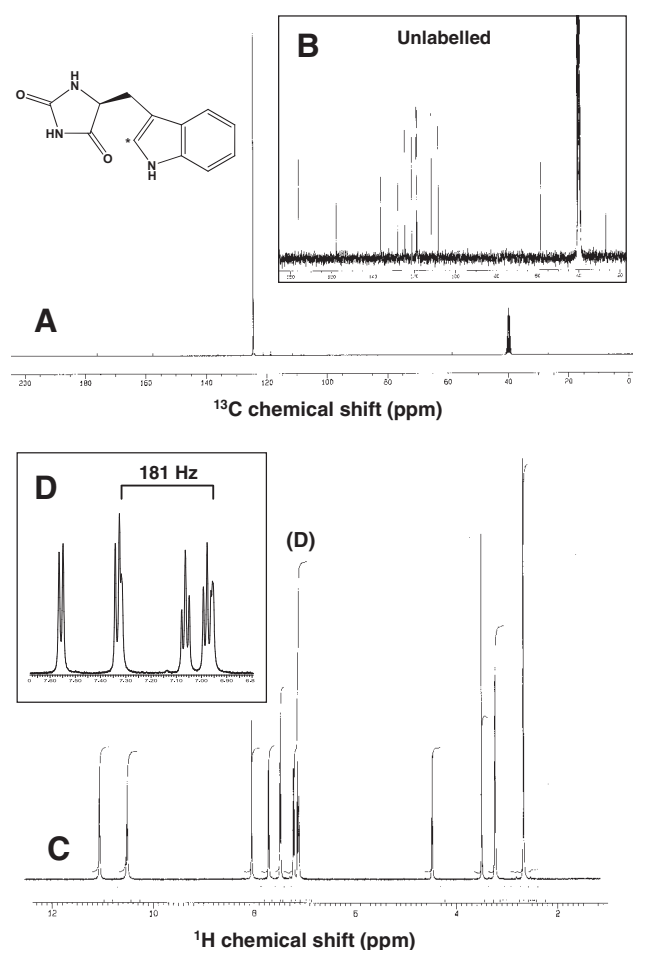


Figure 3. NMR analysis of [indole-2-^{13}C]-*L*-5-indolylmethylhydantoin. (A and B) ^{13}C NMR spectra of [indole-2-^{13}C]-*L*-5-indolylmethylhydantoin and unlabelled *L*-5-indolylmethylhydantoin, respectively, in DMSO-d_6 obtained at 75.48 MHz. (C and D) ^{1}H NMR spectrum of [indole-2-^{13}C]-*L*-5-indolylmethylhydantoin in DMSO-d_6 obtained at 500.23 MHz; D shows an expansion of the indole-H2 signal and its coupling with the ^{13}C label at indole-C2. The asterisk shows the position of the ^{13}C label on the structure of *L*-IMH.

of *L*-BH was performed on a 250-mg scale, but the original reaction mixture included 500 μCi [^{14}C]potassium cyanate. This produced 78 mg of [2-^{14}C]-*L*-5-benzylhydantoin in a chemical yield of 27% from *L*-phenylalanine and with a ^{14}C specific activity of 258.6 μCi/mmol; the radiochemical yield from [^{14}C]potassium cyanate was 21%.

L-5-Indolylmethylhydantoin

Our synthesis of *L*-IMH was based on that described by Suzuki *et al.*[24] for other amino acid hydantoins on a multigram scale, but with major changes and additions to the procedure to tailor it to *L*-IMH and to satisfy our requirements for preparing it with ^{13}C/^{14}C labels and on a smaller scale. On a 200-mg scale, *L*-tryptophan was heated with an excess of potassium cyanate then the solution was acidified to give a colourless fine precipitate of *N*-carbamoyl-*L*-tryptophan; the *N*-carbamoyl amino acid had not been isolated in the reported procedure. The fineness of the precipitate led to significant losses of material on attempts to collect it by filtration, so the precipitate was instead collected and washed by repeated centrifugation and resuspension. Reflux of the precipitate in hydrochloric acid followed by cooling produced colourless crystals of *L*-IMH. The crystals were again collected and washed by centrifugation and the pH was neutralized to remove any residual *N*-carbamoyl-*L*-tryptophan. Care has to be taken here as racemization of amino acid hydantoins occurs under mild alkali conditions.[26,30] Finally, hot filtration and recrystallization from water gave *L*-IMH in 49% yield from *L*-tryptophan. The identity and high purity of the synthesized *L*-IMH was confirmed by melting point, NMR, mass spectrometry and elemental analyses, therefore allowing us to proceed with the labelled versions of *L*-IMH. The use of centrifugation rather than filtration for the collection and washing of crystals not only gave us higher yields but also helped to keep materials more contained in the radiolabelled synthesis.

For solid-state NMR experiments, the target position for a ^{13}C label in *L*-IMH was C-2 in the indole ring; this has a ^{13}C NMR chemical shift of 124.5 ppm, which is in a relatively clear region of the ^{13}C NMR solid-state NMR spectrum of bacterial inner membranes.[32–36] Starting from [indole-2-^{13}C]-*L*-tryptophan, the synthesis was performed as for unlabelled *L*-IMH to give 96 mg of [indole-2-^{13}C]-*L*-indolylmethylhydantoin in a yield of 42% from the amino acid. The ^{13}C NMR spectrum of [indole-2-^{13}C]-*L*-IMH (Figure 3A) shows exclusive ^{13}C-enrichment at C-2 of the indole ring along with a ^{13}C–^{13}C splitting of the signal for

indole-C1 with a coupling constant of 70.1 Hz and long-range ^{13}C–^{13}C splittings of other signals. In the ^{1}H NMR spectrum, the indole-H2 signal at 7.14 ppm shows a ^{1}H–^{13}C coupling constant of 181 Hz (Figure 3C and D).

Radiolabelled *L*-IMH was also required for our transport assays; as with *L*-BH, [^{14}C]potassium cyanate was used to introduce a ^{14}C label at C-2 in the hydantoin ring (Scheme 1). The synthesis was performed as for unlabelled *L*-IMH except that 500 μCi [^{14}C]potassium cyanate was included in the reaction mixture. This produced 102 mg of [2-^{14}C]-*L*-5-indolylmethylhydantoin in a chemical yield of 45% from *L*-tryptophan and with a ^{14}C specific activity of 386.6 μCi/mmol; the radiochemical yield from [^{14}C]potassium cyanate was 35%.

Conclusions

This work has described the first syntheses of ^{13}C- and ^{14}C-labelled 5-benzyl- and 5-indolylmethyl *L*-hydantoins. Robust and straightforward procedures were developed for preparing *L*-BH and *L*-IMH, as highly pure crystalline solids in moderate and consistent yields from 200/250 mg scale reactions and without using chromatography; these were then used to prepare *L*-BH and *L*-IMH with ^{13}C and ^{14}C labels. The successful incorporation and integrity of the ^{13}C labels was confirmed by NMR spectroscopy. The approach is suitable for the synthesis of *L*-BH and *L*-IMH with other labelling patterns or of other amino acid hydantoins with stable isotope labels or radiolabels.

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