

## REVIEW ARTICLE

# Synthesis, NMR analysis and applications of isotope-labelled hydantoins

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**Abstract** This review concerns methods of synthesis, NMR analysis and applications of isotope-labelled hydantoins. The hydantoin moiety is present in natural products and in extraterrestrial ice, indicating this to be an important compound in prebiotic chemistry. Bacterial transport proteins that scavenge hydantoins have been identified, isolated and characterised with isotope-labelling of hydantoins as an essential requirement to achieve this. These are Mhp1 from *Microbacterium liquefaciens* and PucI from *Bacillus subtilis*, transporting 5-aryl-substituted hydantoins and allantoin, respectively. The hydantoin ring is a useful centre in synthetic chemistry, especially for combinatorial chemistry, multicomponent reactions and in diversity-oriented synthesis. It is also found in pharmacologically active molecules, such as the anticonvulsant phenytoin. Hydantoins synthesised with isotope labels include hydantoin itself, allantoin, other 5-monosubstituted derivatives, phenytoin, other 5,5-disubstituted derivatives, *N*-substituted derivatives and other more complex molecules with multiple substituents. Analysis of isotope-containing hydantoins by NMR spectroscopy has been important for confirming purity, labelling integrity, specific activity and molecule conformation. Isotope-labelled hydantoins have been used in a range of biological, biomedical, food and environmental applications including metabolic and *in vivo* tissue distribution studies, biochemical analysis of transport proteins, identification and tissue distribution of drug binding sites, drug metabolism and pharmacokinetic studies and as an imaging agent.

**Keywords:** allantoin; drug binding and metabolism; hydantoins; isotopic labeling; NMR analysis; PET imaging; phenytoin; transport assays

## 1. INTRODUCTION

Hydantoin (IUPAC name imidazolidine-2,4-dione) (**1**) (Figure 1) is a heterocyclic ring system that occurs relatively rarely in nature. The most commonly known natural product with the hydantoin ring is the urea derivative allantoin (5-ureidohydantoin) (**2**) (Figure 1), which is a constituent of urine and a major metabolic intermediate in most types of organisms including bacteria,

fungi, plants and animals. Allantoin is also present in a number of toothpastes, mouth washes, shampoos and cosmetic products and is used in medications that treat skin conditions including acne, impetigo, eczema and psoriasis. Other natural products that contain the hydantoin ring as part of their chemical structure have been isolated from marine sponges [1,2], from a Mediterranean Sea anemone [3] and from the fungus *Fusarium sp* [4]. A fulvic acid polymer isolated from a coastal pond in Antarctica is also suggested to contain a hydantoin ring based on a solid-state NMR <sup>15</sup>N and <sup>13</sup>C{<sup>14</sup>N} chemical shift investigation [5]. Interestingly, the presence of hydantoin in extraterrestrial ice has been demonstrated, indicating this to be an important compound in prebiotic chemistry [6], and a new route for the prebiotic synthesis of hydantoin in water/ice/urea solutions involving the photochemistry of acetylene has been proposed [7].

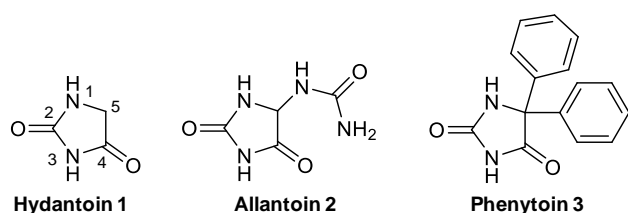


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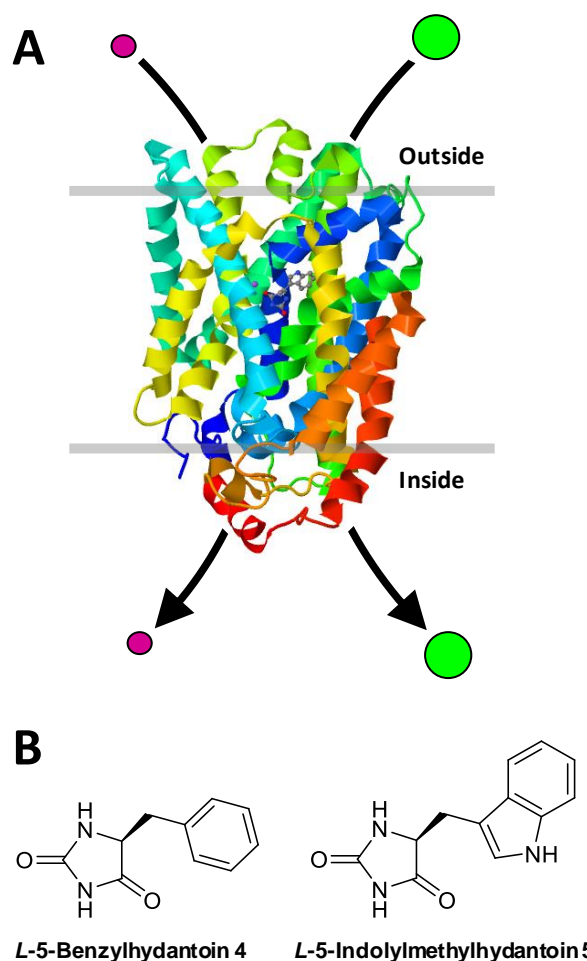
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**Figure 1.** Structures of hydantoin (1) and the common 5-substituted derivatives allantoin (2) and phenytoin (3).

The first reported synthesis of hydantoin was in 1861 by Baeyer [8], but its structure was not assigned correctly until 1870 by Strecker [9]. The chemical properties, methods of synthesis and reactivity of hydantoin and its derivatives have been reviewed extensively [10-15]. For synthetic chemistry applications, hydantoin is a useful centre in combinatorial chemistry [16], multicomponent reactions [17,18] and in diversity-oriented synthesis [19-21]. Hydantoins substituted at the 5-position are precursors to optically pure natural and unnatural  $\alpha$ -amino acids, which is achieved through their chemical or enzymatic hydrolysis [22-30]. They therefore serve as important compounds in the food industry, for example in the production of the artificial sweetener aspartame (*N*-(*L*-aspartyl)-*L*-phenylalanine, 1-methyl ester), which can be synthesised from its constituent *L*- $\alpha$ -amino acids. They are important in the pharmaceutical industry as precursors to optically pure *D*-amino acids [31-33], which are used in the production of certain drugs such as  $\beta$ -lactam antibiotics (e.g. penicillin and amoxicillin) and anticancer agents (e.g. goserelin). The hydantoin moiety itself also forms the basis or is a constituent of a number of pharmacologically active molecules, the most well known being anticonvulsants such as phenytoin (5,5-diphenylhydantoin) (3) (Figure 1) [34-37].

A protein called Mhp1 that promotes the uptake of 5-aryl substituted hydantoins into cells of the Gram positive bacterium *Microbacterium liquefaciens*, serving as part of a salvage pathway for carbon nutrients, has been identified, isolated and purified and its high-resolution crystal structure (Figure 2A) determined in three different conformations (open to outside, occluded with substrate, open to inside), the first for any secondary active transport protein [38-43]. Mhp1 is a member of the widespread nucleobase-cation-symport-1 (NCS1) family of secondary active transport proteins with members in bacteria, fungi and plants [44-52] and has provided a pivotal model for the alternating access mechanism of membrane transport and for the mechanism of ion-coupling [41,42,53-57]. Mhp1 is located in the cytoplasmic cell membrane where it catalyses the inward co-transport of a sodium ion down its concentration gradient and of a hydantoin molecule against its concentration gradient (Figure 2A). This mechanism enables the bacterium to scavenge low concentrations of hydantoin compounds from its environment. The principal transported substrates of Mhp1 are *L*-5-benzylhydantoin (4) and *L*-5-indolylmethylhydantoin (5) (Figure 2B).



**Figure 2.** Hydantoin transport protein, Mhp1, from the Gram positive bacterium *Microbacterium liquefaciens*. **A.** Schematic illustration of the 3.4 Å-resolution crystal structure of Mhp1 determined in complex with the substrate *L*-5-indolylmethylhydantoin [62]. Mhp1 is shown in a cell membrane where it catalyses the inward co-transport of a sodium ion (purple circle) down its concentration gradient and of a hydantoin molecule (green circle) against its concentration gradient. This mechanism enables the bacterium to scavenge low concentrations of hydantoin compounds from its environment for use as sources of carbon and nitrogen. The locations of a sodium ion and of a hydantoin molecule can be seen in the centre of the structure at their respective binding sites. The structure of Mhp1 was drawn using RSCB Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>) file 4D1A using Jmol [63]. **B.** Substrates of the Mhp1 transport protein *L*-5-benzylhydantoin (4) and *L*-5-indolylmethylhydantoin (5).

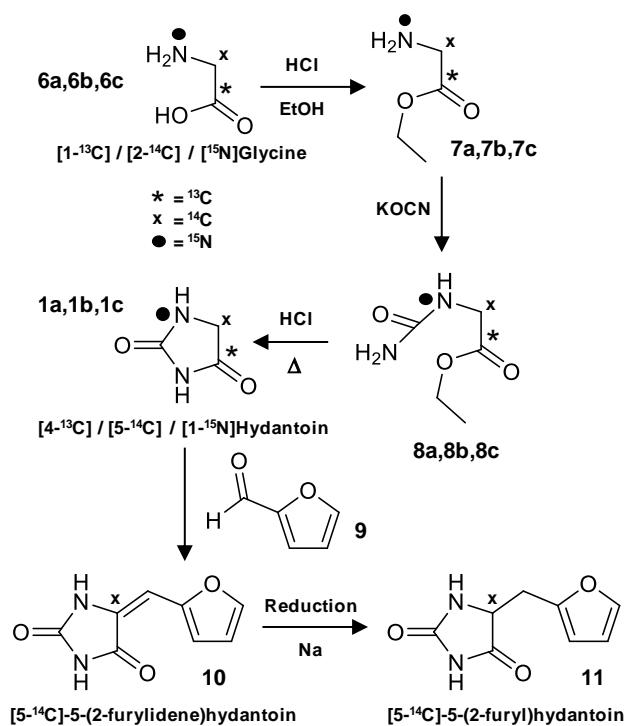
An allantoin transport protein called PucI from *Bacillus subtilis* has recently been isolated, purified and characterised [58]. PucI shares evolutionary relationships with other putative bacterial allantoin permeases, with Mhp1 and with other characterised NCS1 transporters in fungi and plants [58]. Crucial to the success in characterising the ligand recognition, substrate selectivity and transport kinetics of Mhp1 and PucI was synthesis of a number of isotope-labelled hydantoins and their use in biochemical assays [39,58-62]. Analysis of the synthesised isotope-containing hydantoin compounds by NMR spectroscopy was important for confirming their purity and labelling integrity. A significant number and variety of

other isotope-labelled hydantoins have been synthesised and used in a range of biological, biomedical, food and environmental applications. The methods of synthesis, NMR analysis and their applications are the subject of this review.

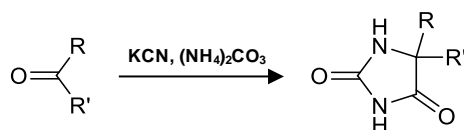
## 2. HYDANTOIN

A classic method for the synthesis of hydantoin uses glycine as the starting compound (Figure 3, Scheme 1). Glycine (**6**) is reacted with ethanol/acid to give the ester (**7**); the amine group is then reacted with potassium cyanate to give the intermediate (**8**), which is cyclised under acid reflux to give hydantoin (**1**). Starting with [ $1\text{-}^{13}\text{C}$ ], [ $2\text{-}^{14}\text{C}$ ] or [ $1\text{-}^{15}\text{N}$ ]glycine (**6a**, **6b**, **6c**) this method has been used to prepare [ $4\text{-}^{13}\text{C}$ ], [ $5\text{-}^{14}\text{C}$ ] and [ $1\text{-}^{15}\text{N}$ ]hydantoin (**1a**, **1b**, **1c**), respectively [64-66]. The [ $4\text{-}^{13}\text{C}$ ]hydantoin and [ $1\text{-}^{15}\text{N}$ ]hydantoin were reacted with indole-3-aldehyde followed by hydrolysis to give *DL*-tryptophan labelled with  $^{13}\text{C}$  at the 1-position [64] or with  $^{15}\text{N}$  at the  $\alpha\text{-N}$  position [66], respectively. The [ $5\text{-}^{14}\text{C}$ ]hydantoin was reacted with furfural (**9**) to give [ $5\text{-}^{14}\text{C}$ ]5-(2-furylidene)hydantoin (**10**)

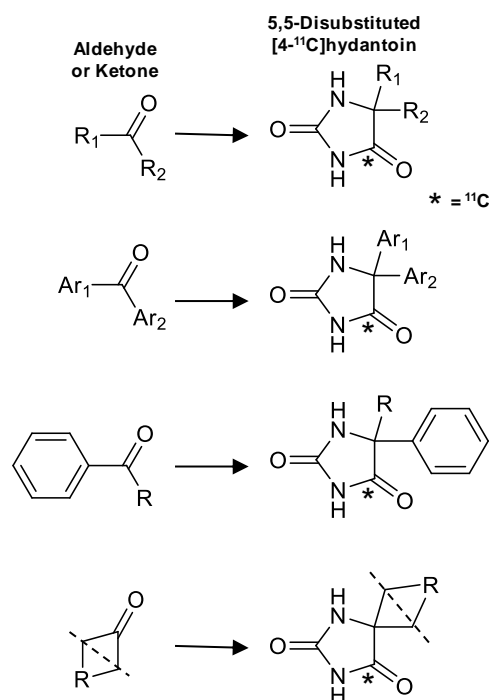
and reduced to [ $5\text{-}^{14}\text{C}$ ]5-(2-furyl)hydantoin (**11**), which was hydrolysed to *DL*-[ $2\text{-}^{14}\text{C}$ ]3-(2'-furyl)-alanine (Figure 3, Scheme 1) [65]. Another classic method for the synthesis of hydantoin, and derivatives thereof substituted at the 5-position, is the Bucherer-Bergs reaction [67-69]. This is the multicomponent reaction of carbonyl compounds (aldehydes or ketones) or cyanohydrins with potassium cyanide and ammonium carbonate to give hydantoins where the chemical groups on the carbonyl compound become the substituents at the 5-position in the hydantoin (Figure 3, Scheme 2). Use of the simplest aldehyde formaldehyde in this reaction does give hydantoin (**1**), but also other products including hydantoic acid and hydantoic amide [70]. Work by Winstead *et al* [71] nicely demonstrates the range of aliphatic, aromatic and cyclic substituted hydantoins that can be produced by the Bucherer-Bergs reaction, which in this case were labelled with the positron emitting carbon-11 at the 4-position by using [ $^{11}\text{C}$ ]potassium cyanide in the reaction (Figure 3, Scheme 3). The  $^{11}\text{C}$ -labelled hydantoins prepared here were used to measure their *in vivo* tissue distribution pattern in dogs.



Scheme 1



Scheme 2



Scheme 3

**Figure 3.** Synthesis of [ $4\text{-}^{13}\text{C}$ ], [ $5\text{-}^{14}\text{C}$ ] and [ $1\text{-}^{15}\text{N}$ ]hydantoin (**1a**, **1b**, **1c**) from [ $1\text{-}^{13}\text{C}$ ], [ $2\text{-}^{14}\text{C}$ ] or [ $1\text{-}^{15}\text{N}$ ]glycine (Scheme 1), the Bucherer-Bergs reaction for synthesis of 5-substituted hydantoins (Scheme 2) and its use in preparing a range of aliphatic, aromatic and cyclic substituted [ $4\text{-}^{11}\text{C}$ ]hydantoins (Scheme 3).

### 3. 5-MONOSUBSTITUTED HYDANTOINS

A number of 5-monosubstituted hydantoins have been synthesised with isotope labels, where the label has been directed to the substituent group and/or to the hydantoin moiety. Starting with [1-<sup>14</sup>C]phenol (**12a**), 5-(4-methoxy)benzylhydantoin (**16**) and 5-(4-hydroxy)benzylhydantoin (**17**) were synthesised with a <sup>14</sup>C label at the 4-position in the benzyl ring (Figure 4, Scheme 4) [72]. The [1-<sup>14</sup>C]phenol (**12a**) was methylated with dimethyl sulphate to give [1-<sup>14</sup>C]anisole (**13**) which was converted to a mixture of *ortho*- and *para*-[4-<sup>14</sup>C]-anisaldehyde (**14**, **15**) by a modified Gattermann reaction and then the separated *para*-form was condensed with hydantoin (**1**) to give [ring-4-<sup>14</sup>C]-5-(4-methoxy)benzyl-hydantoin (**16**) from which the methyl group was removed to give [ring-4-<sup>14</sup>C]-5-(4-hydroxy)benzylhydantoin (**17**). The latter compound was then converted into [ring-4-<sup>14</sup>C]-*DL*-tyrosine. Hydantoins **16** and **17** have also been prepared with a <sup>14</sup>C label at the 6-position (CH<sub>2</sub>) by condensing hydantoin with [1-<sup>14</sup>C]anisaldehyde and used as intermediates in the synthesis of [3-<sup>14</sup>C]-*DL*-tyrosine [73,74]. A similar approach has been used for synthesis of [1-<sup>14</sup>C]-*DL*-tyrosine [75,76]. A tritiated form of 5-indolylmethylhydantoin (**5a**) has been prepared by combining hydantoin (**1**) with indole-3-carboxaldehyde (**18**) and the resultant alkene (**19**) then reduced with tritium gas to give [5,6-<sup>3</sup>H]-5-indolylmethylhydantoin (**5a**) (Figure 4, Scheme 5). This was then converted into [2,3-<sup>3</sup>H]-*DL*-tryptophan [77]. Using a method based on Gaudry's synthesis [78], another <sup>14</sup>C-labelled amino acid, [1-<sup>14</sup>C]-*L*-lysine, was prepared *via* the intermediates 5-hydroxyhydantoin and 5-bromobutylhydantoin [79]. A hydantoin with a 123m-tellurium labelled 5-substituent (**23**) has been synthesised starting from [<sup>123m</sup>Te]diphenyl ditelluride (**20**) (Figure 4, Scheme 6). This compound was reduced by sodium borohydride to generate [<sup>123m</sup>Te]phenyltellurol (**21**), which was then reacted with 5-(β-bromoethyl)hydantoin (**22**) to give [<sup>123m</sup>Te]-5-[β-(phenyltelluro)ethyl]hydantoin (**23**). The hydantoin was hydrolysed to [<sup>123m</sup>Te]-*DL*-α-amino-γ-(phenyltelluro)-butyric acid, which was used as a potential pancreatic imaging agent [80].

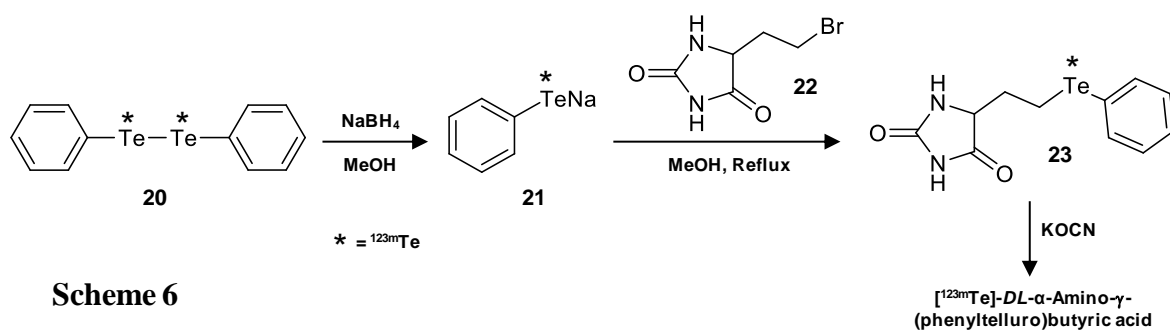
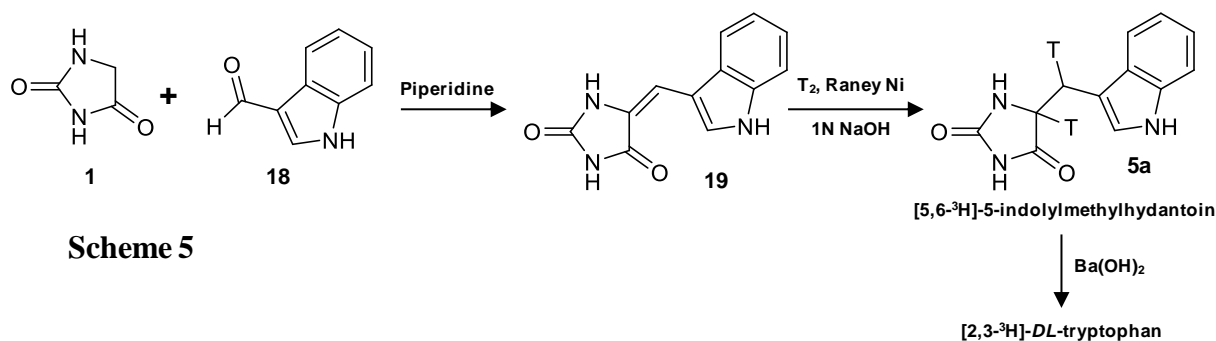
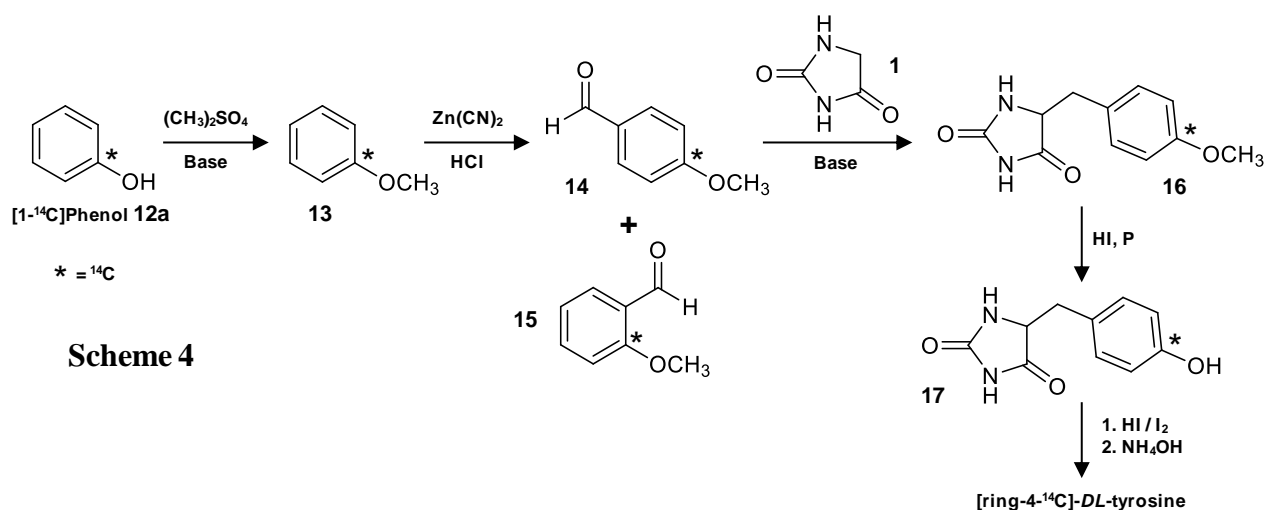
*DL*-Allantoin (**2**) has been synthesised with <sup>13</sup>C or <sup>14</sup>C labels using urea as the source of the label (Figure 5, Scheme 7) [59]. This synthesis began by reduction of parabanic acid (**24**) to give 5-hydroxyhydantoin, which was then treated with thionyl chloride to give 5-chlorohydantoin (**25**). Reaction with [<sup>13</sup>C]- or [<sup>14</sup>C]urea (**26a**, **26b**) produced the labelled forms of *DL*-allantoin (**2a**, **2b**). In this case, NMR analysis was not only important for demonstrating high purity of the labelled product, but the <sup>13</sup>C NMR spectrum of **2a** (Figure 6A) also revealed a partial scrambling of the <sup>13</sup>C label from the ureido group (157.7 ppm) to the C-2 position (157.1 ppm) [59]. This confirmed a rearrangement of allantoin in solution *via* a putative bicyclic intermediate [81] and so **2a** was assigned as *DL*-[H<sub>2</sub>N<sup>13</sup>CO/<sup>13</sup>C-2]allantoin. The <sup>14</sup>C-labelled allantoin (**2b**)

synthesised by the same method was therefore also assigned as *DL*-[H<sub>2</sub>N<sup>14</sup>CO/<sup>14</sup>C-2]allantoin [59]. The <sup>14</sup>C-labelled compound has been used in whole cell uptake assays with the transport proteins Mhp1 and PucI in experiments to define their substrate selectivities, ligand recognition and transport kinetics (Figure 7) [58,62]. Crucially, these measurements provided the first experimental evidence to demonstrate that PucI is a medium-affinity transporter of allantoin. The Mhp1 substrates *L*-5-benzylhydantoin (**4**) and *L*-5-indolylmethylhydantoin (**5**) have been synthesised containing both <sup>13</sup>C and <sup>14</sup>C labels (Figure 5) for use in solid-state NMR measurements of ligand binding (unpublished) and in whole cell transport assays, respectively, with the Mhp1 protein [60,62]. These compounds were prepared from the appropriate *L*-α-amino acid (phenylalanine or tryptophan) by reaction with potassium cyanate under acidic conditions to give the *L*-carbamoyl-*L*-α-amino acid, which was then cyclised to give the 5-substituted *L*-hydantoin (Figure 5, Scheme 8). This is based on the classic Urech hydantoin synthesis [82]. [6-<sup>13</sup>C]-*L*-5-Benzylhydantoin (**4a**) was prepared from [3-<sup>13</sup>C]-*L*-phenylalanine and [indole-2-<sup>13</sup>C]-*L*-5-indolylmethylhydantoin (**5b**) was prepared from [indole-2-<sup>13</sup>C]-*L*-tryptophan. NMR analysis of the <sup>13</sup>C-labelled compounds was important for confirming both high purity and labelling integrity [60]. The <sup>13</sup>C NMR spectrum of [6-<sup>13</sup>C]-*L*-5-benzylhydantoin (**4a**) confirmed <sup>13</sup>C enrichment exclusively in the C-6 position at 36.8 ppm and the <sup>1</sup>H NMR spectrum showed a splitting of the H-6 methylene signal at 2.94 ppm with a coupling constant of 129 Hz due to the directly attached <sup>13</sup>C label (Figure 6B). Similarly, the <sup>13</sup>C NMR spectrum of [indole-2-<sup>13</sup>C]-*L*-5-indolylmethylhydantoin (**5b**) confirmed <sup>13</sup>C enrichment exclusively in the indole-C2 position at 124.5 ppm and the <sup>1</sup>H NMR spectrum showed a splitting of the indole-H2 signal at 7.14 ppm with a coupling constant of 181 Hz due to the directly attached <sup>13</sup>C label (Figure 6C). The <sup>14</sup>C-labelled versions of the compounds (**4b**, **5c**) were prepared by using [<sup>14</sup>C]potassium cyanate in the reaction to introduce the label at C-2 in the hydantoin ring [60]. These <sup>14</sup>C-compounds have been used in whole cell uptake assays with Mhp1 that have been crucial in defining its substrate selectivity, ligand recognition and quantitation of ligand binding and in screening the transport activities of Mhp1 mutants (Figure 7). This work also identified a novel inhibitor of Mhp1, 5-(2-naphthylmethyl)hydantoin, which was itself synthesised with a <sup>14</sup>C label at the C-2 position using the same method (Figure 7) [62].

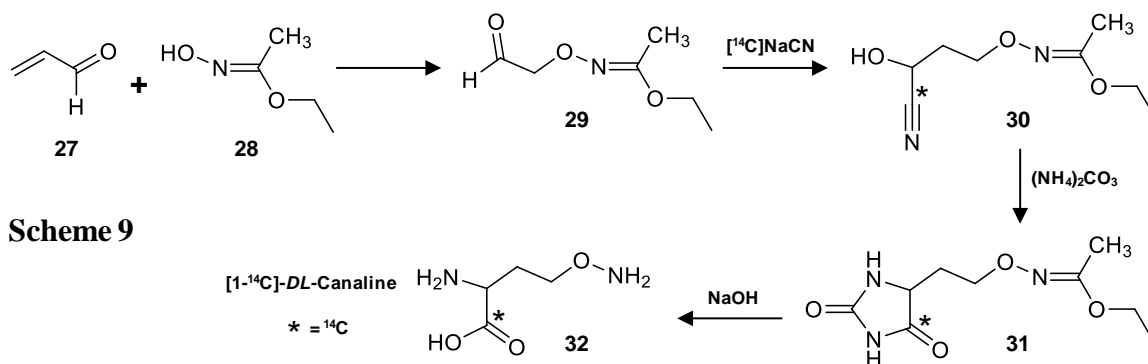
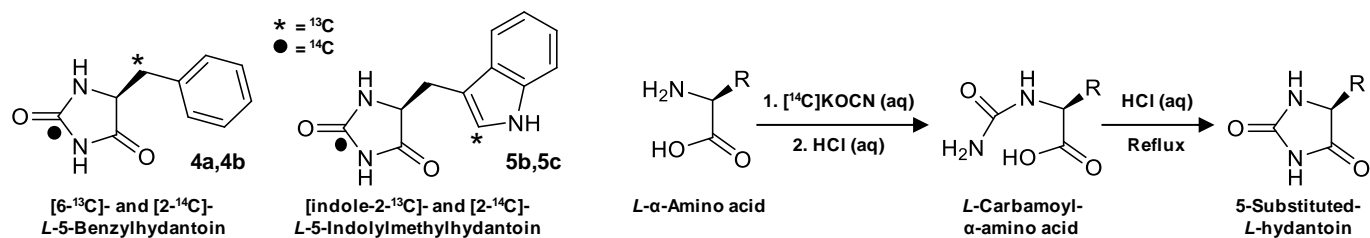
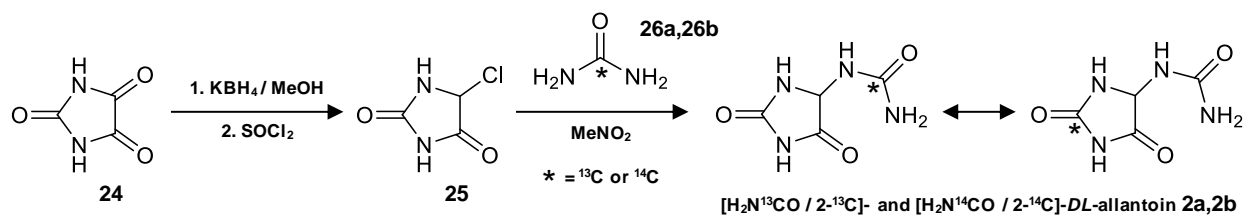
A hydantoin derivative of *DL*-canaline has been synthesised with a <sup>14</sup>C label at C-4 in the hydantoin ring (**31**) as an intermediate in the production of [<sup>14</sup>C]-*DL*-canaline (**32**) itself, which is a structural analog of ornithine (Figure 5, Scheme 9) [83]. The synthesis began by reacting acrolein (**27**) and ethyl *N*-hydroxyacetimidate (**28**) to give ethyl *N*-[3-oxopropoxy]acetimidate (**29**), which is converted into the nitrile (**30**) using [<sup>14</sup>C]sodium cyanide.

The nitrile was cyclised with ammonium carbonate to give the  $^{14}\text{C}$ -labelled hydantoin (**31**). Heating of the hydantoin with sodium hydroxide afforded [1- $^{14}\text{C}$ ]-DL-canaline (**32**),

which was intended for use in evaluating its capacity to support amino acid biosynthesis by a seed-eating beetle.

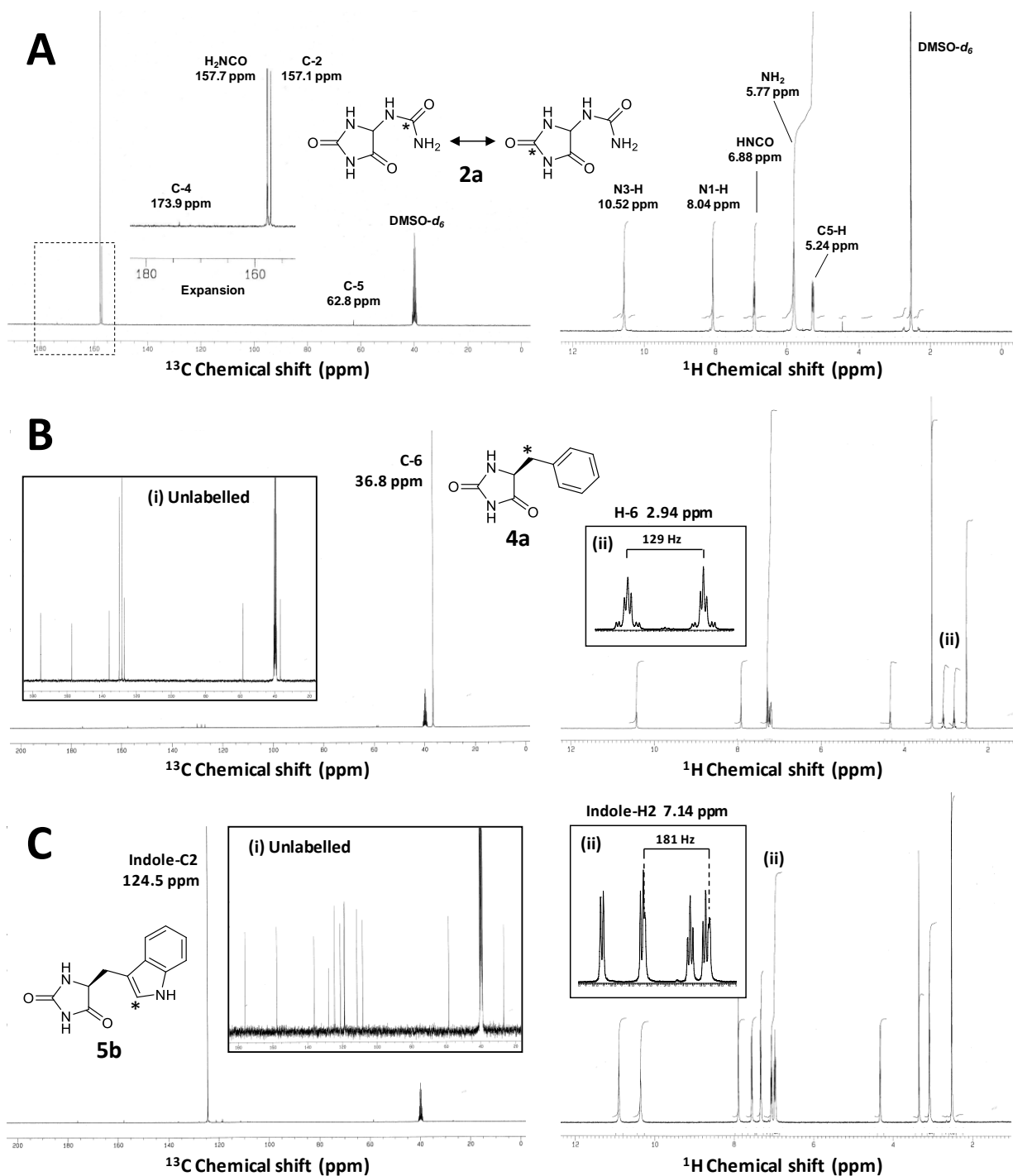


**Figure 4.** Synthesis of the 5-substituted hydantoins [ring-4- $^{14}\text{C}$ ]-5-(4-methoxy)benzylhydantoin (**16**) and [ring-4- $^{14}\text{C}$ ]-5-(4-hydroxy)benzylhydantoin (**17**) (**Scheme 4**), [5,6- $^3\text{H}$ ]-5-indolylmethylhydantoin (**5a**) (**Scheme 5**) and [ $^{123\text{m}}\text{Te}$ ]-5-[β-(phenyltelluro)ethyl]hydantoin (**23**) (**Scheme 6**).

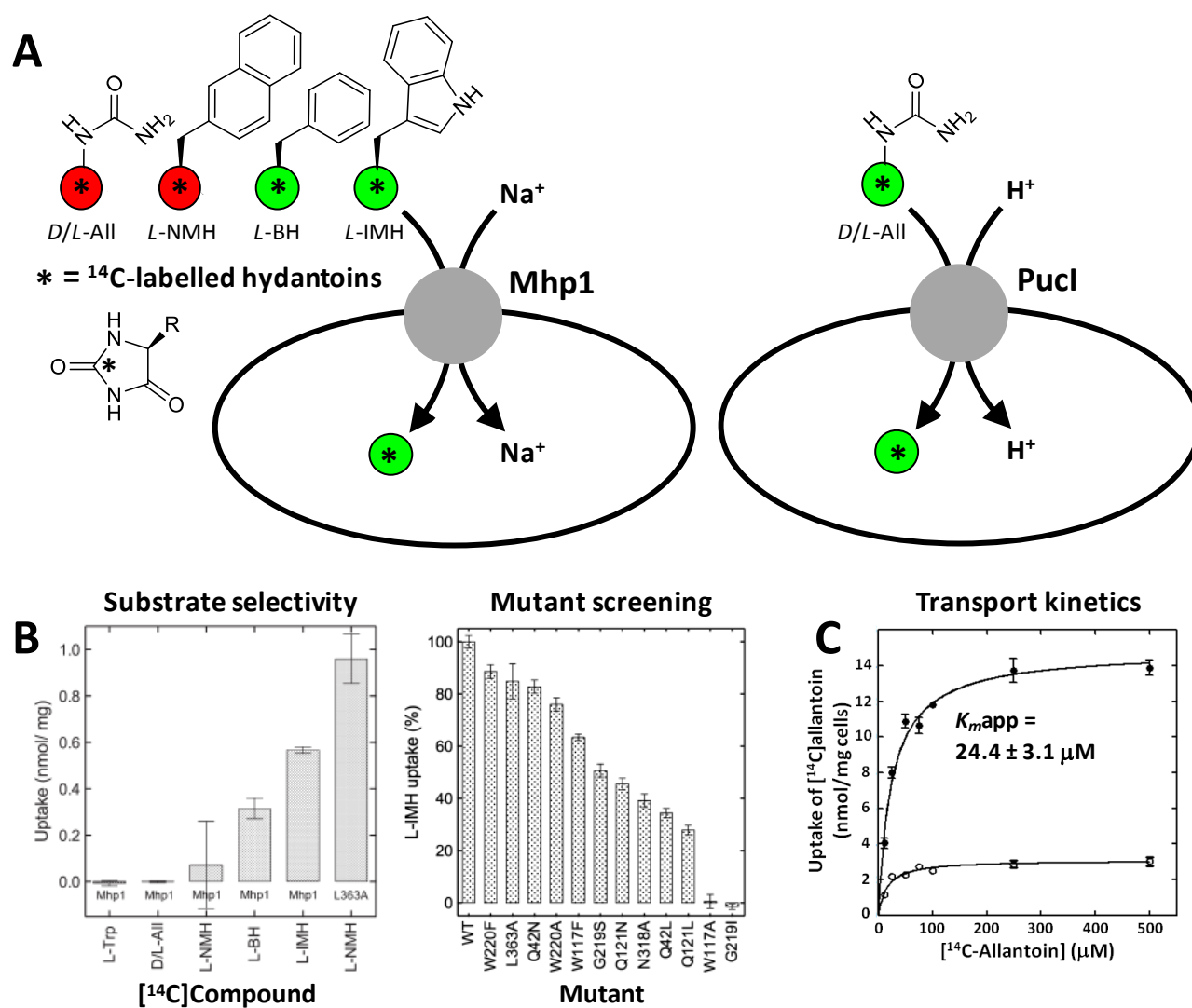


**Figure 5.** Synthesis of the 5-substituted hydantoin *DL*-[H<sub>2</sub>N<sup>13</sup>CO/<sup>13</sup>C-2]allantoin (**2a**) and *DL*-[H<sub>2</sub>N<sup>14</sup>CO/<sup>14</sup>C-2]allantoin (**2b**) (**Scheme 7**), [6-<sup>13</sup>C]-*L*-5-benzylhydantoin (**4a**), [2-<sup>14</sup>C]-*L*-5-benzylhydantoin (**4b**), [indole-2-<sup>13</sup>C]-*L*-5-indolylmethylhydantoin (**5b**), [2-<sup>14</sup>C]-*L*-5-indolylmethylhydantoin (**5c**) (**Scheme 8**) and a hydantoin derivative of *DL*-canaline with a <sup>14</sup>C label at C-4 (**31**) (**Scheme 9**).





**Figure 6.** NMR analysis of  $^{13}\text{C}$ -labelled 5-substituted hydantoin. **A.**  $^{13}\text{C}$  (left) and  $^1\text{H}$  (right) NMR spectra of  $DL$ -[ $\text{H}_2\text{N}^{13}\text{CO}/^{13}\text{C}$ -2]allantoin (**2a**) in  $\text{DMSO}-d_6$  obtained using a 300 MHz magnet; inset is an expansion of a region of the  $^1\text{H}$  NMR spectrum (dotted line) containing the  $^{13}\text{C}$ -enriched carbonyl signals  $\text{H}_2\text{N}^{13}\text{CO}$  and  $^{13}\text{C}$ -2. **B.**  $^{13}\text{C}$  (left) and  $^1\text{H}$  (right) NMR spectra of  $[6-^{13}\text{C}]$ - $L$ -5-benzylhydantoin (**4a**) in  $\text{DMSO}-d_6$  obtained using a 300 MHz and 500 MHz magnet, respectively; inset are a  $^{13}\text{C}$  NMR spectrum of unlabelled  $L$ -5-benzylhydantoin (i) and an expansion of a region of the  $^1\text{H}$  NMR spectrum containing signals for the H-6 methylene position (ii). **C.**  $^{13}\text{C}$  (left) and  $^1\text{H}$  (right) NMR spectra of  $[\text{indole-2-}^{13}\text{C}]$ - $L$ -5-indolylmethylhydantoin (**5b**) in  $\text{DMSO}-d_6$  obtained using a 300 MHz and 500 MHz magnet, respectively; inset are a  $^{13}\text{C}$  NMR spectrum of unlabelled  $L$ -5-indolylmethylhydantoin (i) and an expansion of a region of the  $^1\text{H}$  NMR spectrum containing signals for the indole-H2 position (ii). This figure was constructed using the results of Patching [59,60]; copyright © 2009, 2010 by John Wiley & Sons, Ltd.



**Figure 7. Whole cell uptake assays for the bacterial transport proteins Mhp1 and Pucl using  $^{14}\text{C}$ -labelled hydantoin.** **A.** Schematic illustration of an assay measuring the uptake of  $^{14}\text{C}$ -labelled hydantoin into energised *Escherichia coli* cells with amplified expression of the transport proteins Mhp1 from *Microbacterium liquefaciens* (left) and Pucl from *Bacillus subtilis* (right). The  $^{14}\text{C}$ -labelled hydantoin tested with sodium-dependent Mhp1 and with proton-dependent Pucl are D/L-allantoin (D/L-AII, **2b**), L-5-(2-naphthylmethyl)hydantoin (L-NMH), L-5-benzylhydantoin (L-BH, **4b**) and L-5-indolylmethylhydantoin (L-IMH, **5c**). The colours represent transported compounds (green) and non-transported compounds (red). **B.** Results for characterising the substrate selectivity of Mhp1 using radiolabelled L-tryptophan (L-Trp) and the  $^{14}\text{C}$ -labelled hydantoin listed above (left) and for screening mutants of Mhp1 for transport of  $^{14}\text{C}$ -L-IMH (**5c**) compared with wild-type (right). **C.** Results for the concentration-dependence of initial rate  $^{14}\text{C}$ -D/L-allantoin (**2b**) uptake into cells that were uninduced (open circles) or induced (closed circles) for expression of Pucl. The data were fitted to the Michaelis-Menten equation to derive the given rate constant ( $K_{m,app}$ ) for Pucl-mediated transport. Pictures in B were reproduced from Simmons *et al* (2014) [62]; copyright © 2014 by the authors. Results in C were reproduced from Ma *et al* (2016) [58]; copyright © 2016 by the authors.

#### 4. PHENYTOIN AND DERIVATIVES

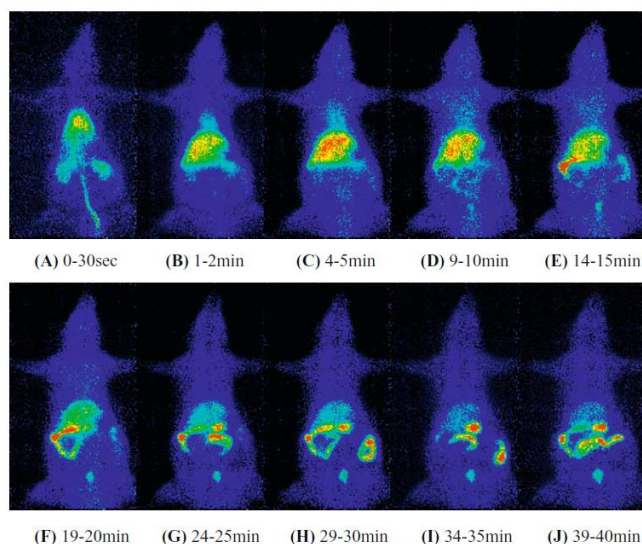
The hydantoin that has been isotope-labelled and used in biomedical applications with highest proliferation is the anticonvulsant drug phenytoin (5,5-diphenylhydantoin) (**3**). A wide range of different isotope labels, labelling patterns and synthetic routes have been used with phenytoin and with derivatives of phenytoin.

Phenytoin with all three carbon positions in the hydantoin ring  $^{13}\text{C}$ -labelled has been synthesised with  $^{13}\text{C}$ carbon dioxide and  $^{13}\text{C}$ urea as the sources of the

labels (Figure 8, Scheme 10) [84]. Reaction of the Grignard reagent phenylmagnesium bromide (**33**) with  $^{13}\text{C}$ carbon dioxide to give  $^{13}\text{C}$ benzoic acid (**34**) was followed by thionyl chloride treatment to give  $^{13}\text{C}$ benzoyl chloride (**35a**) and then reduction to give  $^{13}\text{C}$ benzaldehyde (**36a**). Coupling of two benzaldehyde molecules by reaction with sodium cyanide gave  $^{13}\text{C}$ benzoin (**37a**), which was reacted with  $^{13}\text{C}$ urea (**26a**) to give [2,4,5- $^{13}\text{C}$ ]-5,5-diphenylhydantoin (**3a**). In this case the compound was synthesised for use as a stable-isotope



labelled biomedical tracer using mass spectrometry for detection; this was prior to the availability of a radiolabelled form of phenytoin. Later, both Emran *et al* [85] and Iida *et al* [86] used the same reaction of benzoin (37b) with  $^{11}\text{C}$ - or  $^{13}\text{C}$ -labelled urea (26a, 26b) to produce [2- $^{11}\text{C}$ ]- or [2- $^{13}\text{C}$ ]-5,5-diphenylhydantoin (3b, 3c), respectively (Figure 8, Scheme 11). [2- $^{11}\text{C}$ ]-5,5-Diphenylhydantoin (3c) has also been prepared by reacting 2-amino-2,2-diphenylacetamide (39) with [ $^{11}\text{C}$ ]phosgene (38a) (Figure 8, Scheme 11) [87]. The Bucherer-Bergs reaction has been used with benzophenone (40a) and [ $^{14}\text{C}$ ]potassium cyanide to produce [4- $^{14}\text{C}$ ]-5,5-diphenylhydantoin (3d) and the same method also used to prepare its major metabolite [4- $^{14}\text{C}$ ]-5-(4-hydroxyphenyl)-5-phenylhydantoin (42) (Figure 8, Scheme 12) [88]. A modification of the same reaction with [ $^{11}\text{C}$ ]hydrogen cyanide was also used to produce 5,5-diphenylhydantoin and 5-(4-hydroxyphenyl)-5-phenylhydantoin with an  $^{11}\text{C}$  label at the 4-position and these were used in measurements of their *in vivo* distribution [89]. The brain and whole-body pharmacokinetics of the  $^{11}\text{C}$ -labelled 5,5-diphenylhydantoin in rats has been evaluated using a planar positron imaging system (Figure 9), which demonstrated a difference in the brain distribution of the compound between intravenous and duodenal administration [90].

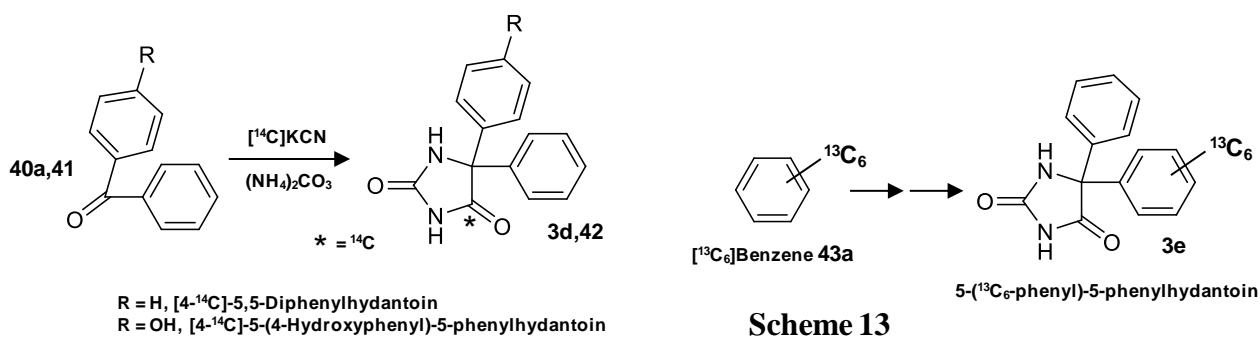
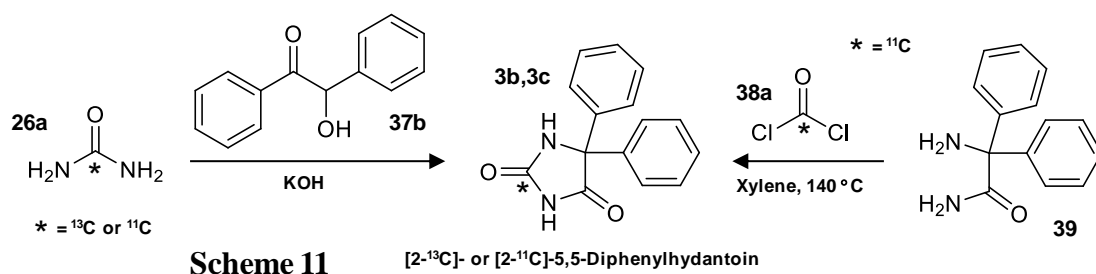
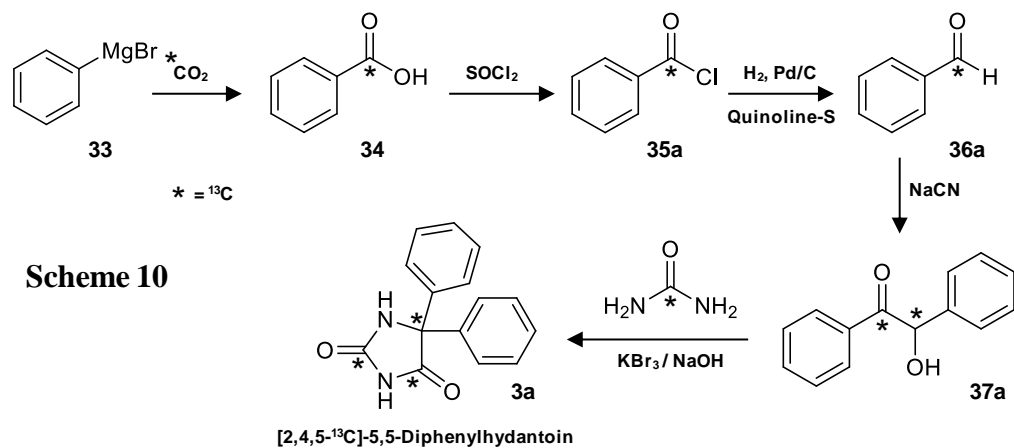


**Figure 9** (Figure 8 is on page 16). **Whole-body imaging of  $^{11}\text{C}$ -labelled 5,5-diphenylhydantoin (DPH) injected into tail vein of rat using planar positron imaging.** A tracer amount of  $^{11}\text{C}$ -DPH (1.5  $\mu\text{g}$ , approximately 5 MBq/250 g) was injected into the tail vein and scans were performed over a total period of 40 minutes. Summation images were created for the first 30 seconds (A) and for the given 1 minute intervals (B-J) to show the accumulation and changes in distribution of  $^{11}\text{C}$ -DPH over time. This figure was reproduced with permission from Hasegawa *et al* (2008) [90]; copyright © 2008 by The Japanese Society of Nuclear Medicine.

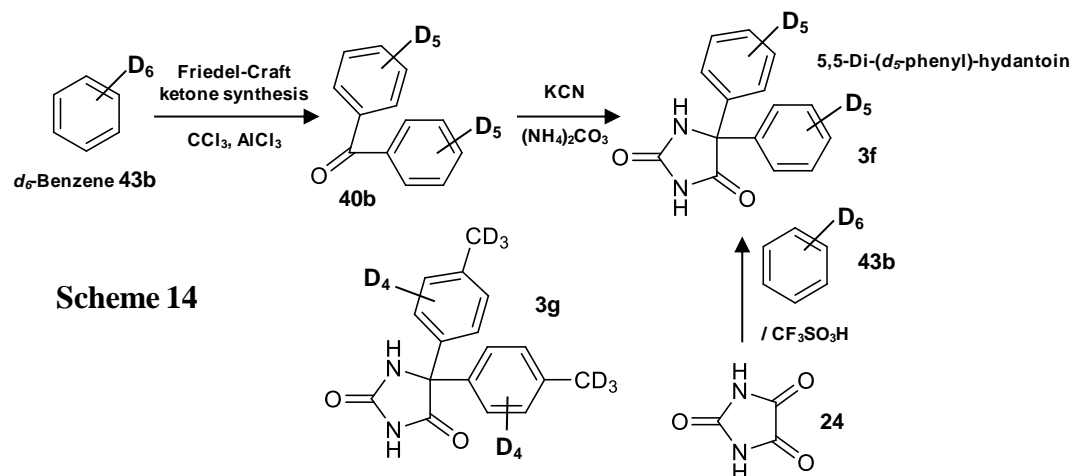
[ $^{13}\text{C}_6$ ]Benzene (43a) has been subject to Friedel-Crafts reaction and then followed by Bucherer-Bergs reaction to produce 5-( $^{13}\text{C}_6$ -phenyl)-5-phenylhydantoin (3e) (Figure 8, Scheme 13) [91]. Using a similar approach but starting with  $d_6$ -benzene (43b), a deuterated form of phenytoin has

been synthesised with all aromatic hydrogen atoms replaced by deuterium (3f) (Figure 8, Scheme 14) [92]. The same compound has also been produced more directly by reacting parabanic acid (24) with  $d_6$ -benzene (43b) and triflic acid (Figure 8, Scheme 14) [93]. This work also prepared a number of deuterated derivatives of phenytoin, for example 3g. Phenytoin with deuteration of just one phenyl ring has been synthesised by Grignard production from  $d_6$ -benzene (43b) and coupling with benzoylformaldehyde (46) to give  $d_5$ -benzoin (37c) which was converted into  $d_5$ -benzil (47) and then condensed with urea (26c) to give 5-( $d_5$ -phenyl)-5-phenylhydantoin (3h) (Figure 10, Scheme 15) [94]. This compound was used as a probe in metabolic studies and as an internal standard for combined GC-MS-computer analyses of body fluid extracts. The synthesis of the 3-hydroxyphenyl and 4-hydroxyphenyl metabolites of phenytoin labelled with deuterium in either of the two phenyl rings (48a, 48b, 49a, 49b) and of 5-( $d_5$ -phenyl)-5-( $d_4$ -*p*-hydroxyphenyl)-hydantoin (48c) (Figure 10) has been achieved by coupling the appropriate labelled and unlabelled components in the production of benzophenone followed by its use in a Bucherer-Bergs reaction (Figure 10, Scheme 16) [95]. The synthesis of phenytoin with a deuterium or tritium atom exclusively at the *para* position of both phenyl rings has been achieved by Bucherer-Bergs reaction with *p*-bromobenzophenone (50) to give 5,5-di-(4-bromophenyl)hydantoin (51) which was then reduced in the presence of deuterium oxide or tritium gas, replacing the bromines to give 5,5-di-(4- $^2\text{H}$ -phenyl)hydantoin (3i) [96] or 5,5-di-(4- $^3\text{H}$ -phenyl)-hydantoin (3j) [97], respectively (Figure 10, Scheme 17). In the case of the deuterated form, for materials with isotopic content up to 95%, excellent agreement was found between the data obtained by mass spectrometry and  $^{13}\text{C}$ -nuclear magnetic resonance operated in a NOE-suppression mode. Phenytoin has also been tritiated directly using  $^3\text{H}_2\text{O}$  and platinum catalyst (from the dioxide and sodium borohydride) and the pattern of labelling was defined by  $^3\text{H}$  NMR spectroscopy [98].

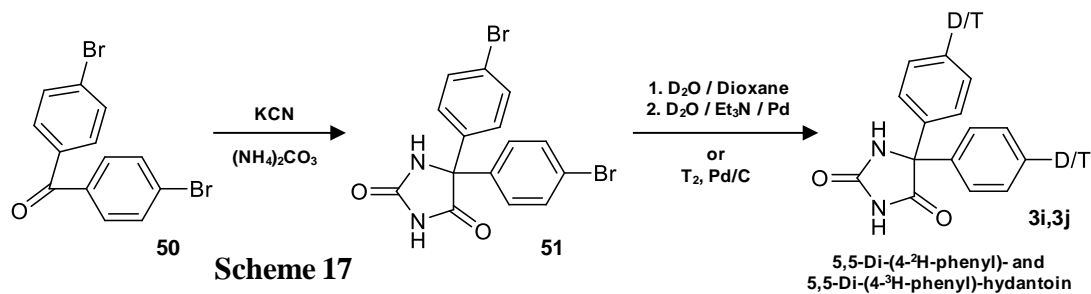
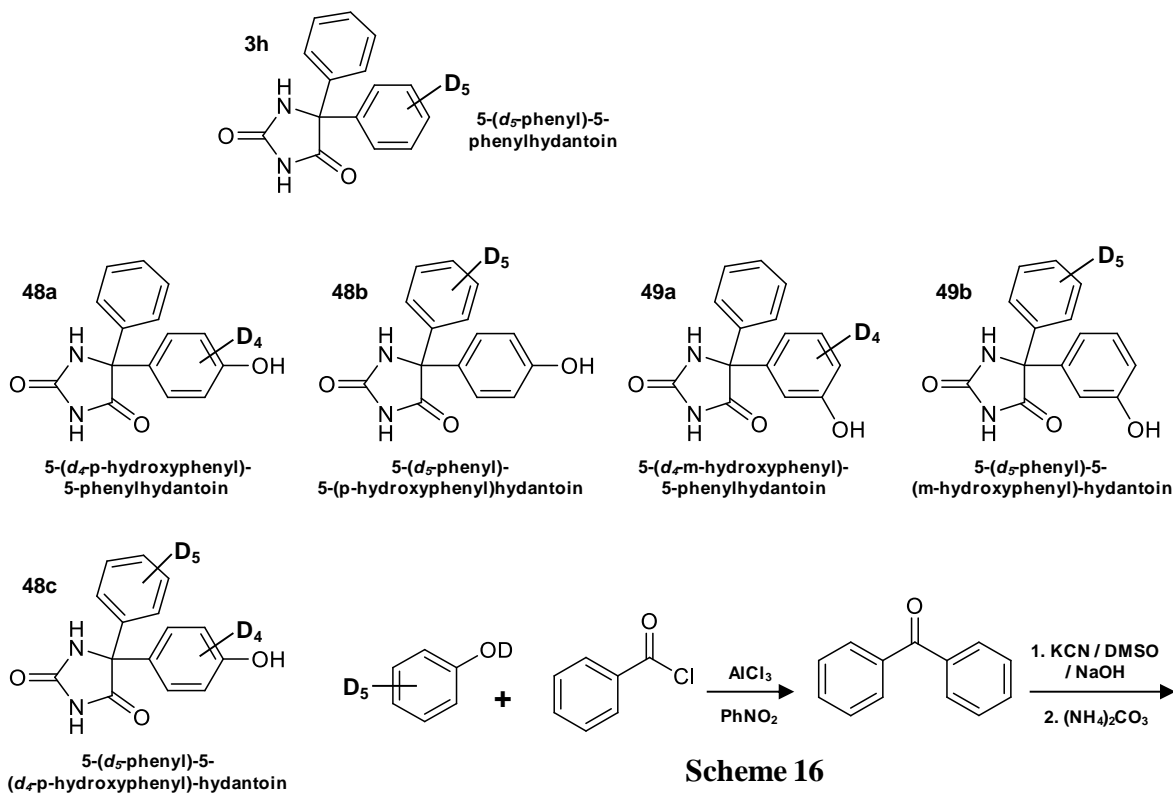
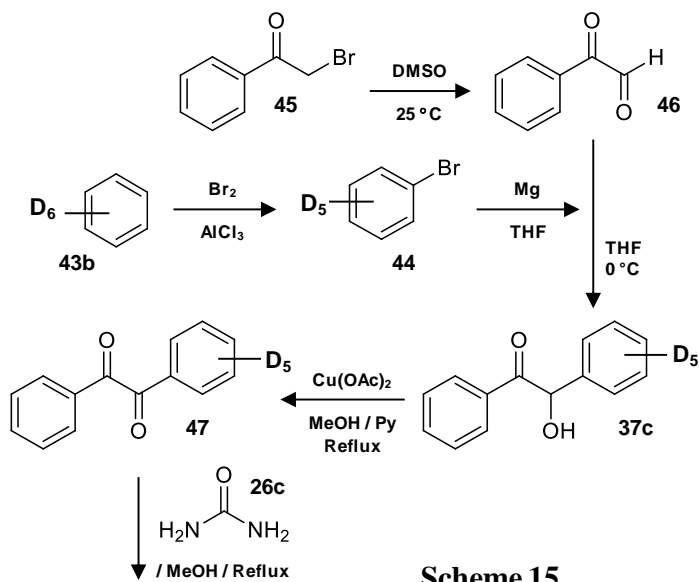
An elegant seven-step synthesis has been used to prepare the separate enantiomers of phenytoin with a single deuterium atom at the *ortho*-position in only one of the phenyl groups (Figure 11, Scheme 18) [99]. The bromine atom of 3-bromoanisole (52) was substituted for deuterium by reduction with deuterium gas and the resultant [3- $^2\text{H}$ ]methoxybenzene (53) was coupled with benzoyl chloride (35b) to give the ketone (54). Bucherer-Burgs reaction produced 5-([2- $^2\text{H}$ ]4-methoxyphenyl)-5-phenylhydantoin (55) from which the methyl group was then removed. The enantiomers of 5-([2- $^2\text{H}$ ]4-hydroxyphenyl)-5-phenylhydantoin (48e, 48f) were separated with brucine resolution [100] and then derivatised at the hydroxyl position with 5-chloro-1-phenyl-1*H*-tetrazole (56), which was removed under reduction conditions to give (*R*)-5-[2- $^2\text{H}$ ]phenyl-5-phenylhydantoin (3k) and (*S*)-5-[2- $^2\text{H}$ ]phenyl-5-phenylhydantoin (3l).



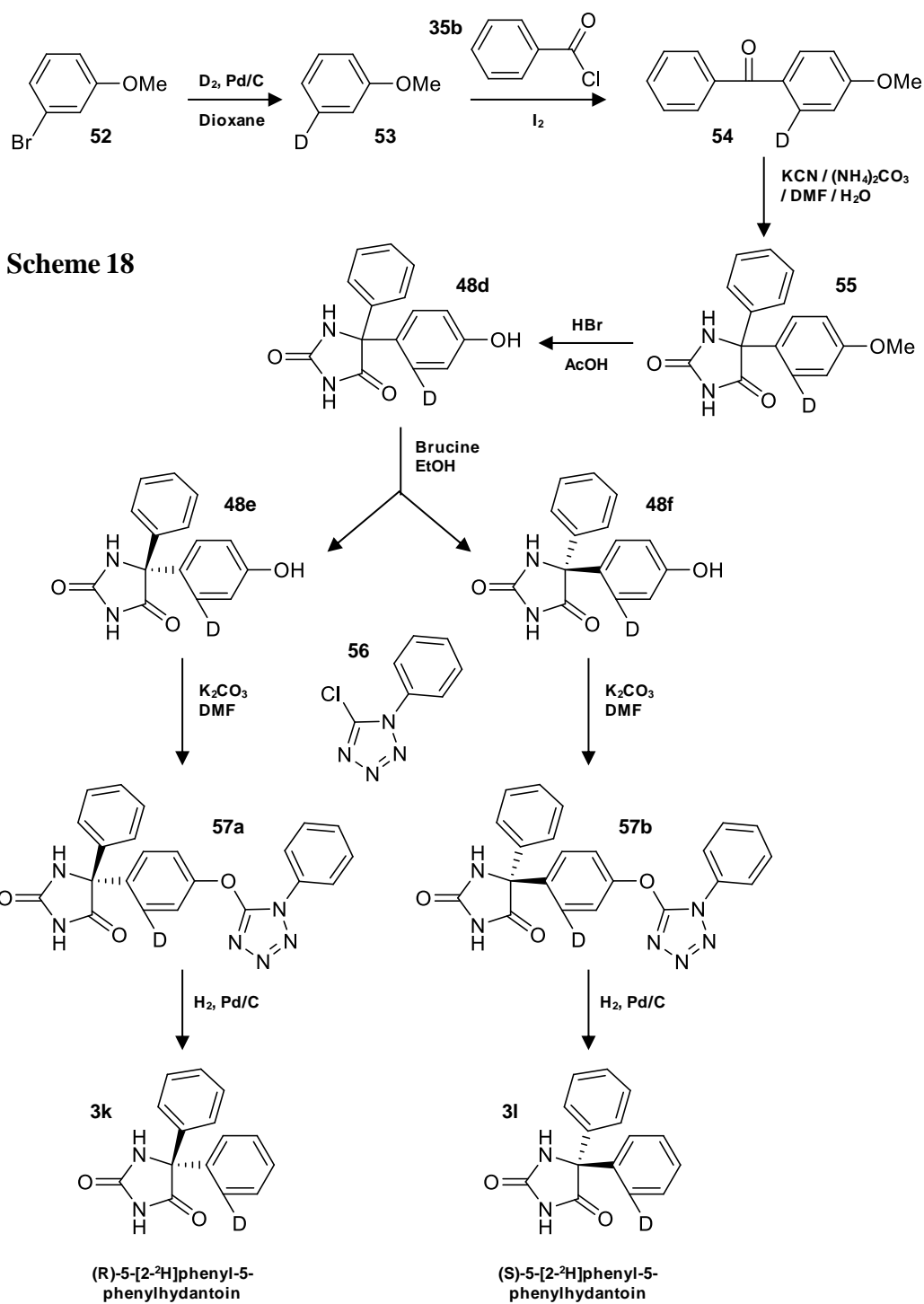
**Scheme 12**



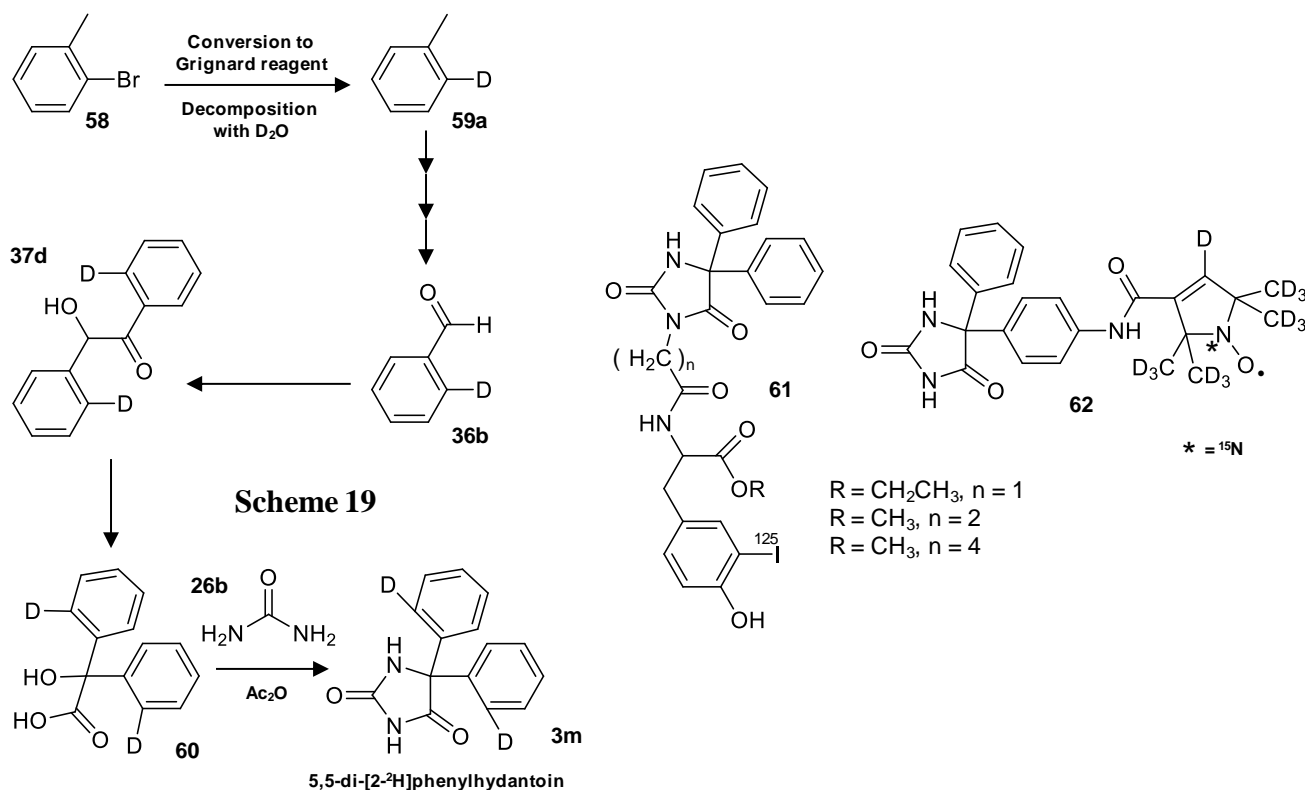
**Figure 8.** Synthesis of isotope-labelled versions of phenytoin (5,5-diphenylhydantoin) (3) (Schemes 10-14).



**Figure 10.** Synthesis of deuterated and tritiated versions of phenytoin (5,5-diphenylhydantoin) (3) (Schemes 15-17).



**Figure 11.** Synthesis of the separate enantiomers of 5-[2-<sup>2</sup>H]phenyl-5-phenylhydantoin (3k, 3l) (Scheme 18).



**Figure 12.** Synthesis of 5,5-di-[2-<sup>2</sup>H]phenylhydantoin (**3m**) (Scheme 19) and structures of phenytoin derivatives with <sup>125</sup>I (**61**) and <sup>15</sup>N, <sup>2</sup>H labels (**62**).

A synthesis of phenytoin with a deuterium atom at the *ortho*-position on both phenyl groups has also been achieved (Figure 12, Scheme 19) [101]. 2-Bromotoluene (**58**) was converted to the Grignard reagent and then decomposed with deuterium oxide to give [2-<sup>2</sup>H]toluene (**59a**) which was converted in three steps to [2-<sup>2</sup>H]benzaldehyde (**36b**). This was converted into the dideuterobenzoin (**37d**) and then dideuterobenzil which was rearranged to the dideuterobenzilic acid (**60**) before condensing with urea (**26b**) to give 5,5-di-[2-<sup>2</sup>H]phenylhydantoin (**3m**). A deuterium atom at the *ortho*-position in phenytoin is more stable to metabolism than at other positions and therefore more suitable to use in metabolic studies that are followed by mass spectrometry.

A number of isotope-labelled derivatives of phenytoin with more complex substituents have also been synthesised. These include a series of <sup>125</sup>I-labelled N<sub>3</sub>-substituted acetamido-tyrosine ethyl ester derivatives (**61**) used as iodinated tracers in a radioimmunoassay for phenytoin [102,103] and a [<sup>15</sup>N, <sup>2</sup>H]<sub>13</sub>2,2,5,5-tetramethylpyrrolidine-1-oxyl-3-carboxylic acid derivative with the substituent at a phenyl *para*-position (**62**) also containing a spin label for detection by electron paramagnetic resonance (EPR) spectroscopy (Figure 12) [104].

## 5. OTHER 5,5-DISUBSTITUTED HYDANTOINS

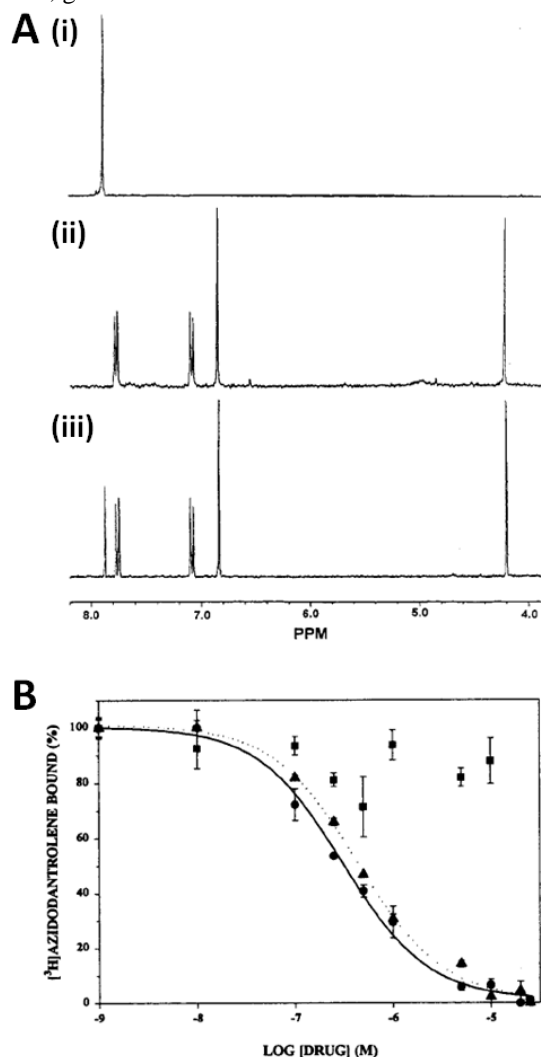
A number of other 5,5-disubstituted hydantoins have been synthesised and used in a range of different applications. A <sup>13</sup>C-labelled benzylated hydantoin intermediate (**66**) has been used in the synthesis of the  $\alpha$ -adrenergic agonist  $\alpha$ -methyldopa with a <sup>13</sup>C label at the benzylic position (**67**) (Figure 13, Scheme 20) [105]. A lithio derivative of 3,4-dibenzoyloxybenzene (**64**) was subject to carbonation with [<sup>13</sup>C]carbon dioxide and the resultant benzoic acid (**65**) was reduced with lithium aluminium hydride. Oxidation with chromium trioxide gave the benzaldehyde which was condensed with nitroethane to form the phenylnitropropene. Reduction of the phenylnitropropene gave the corresponding phenyl-2-propanone which was converted to the hydantoin (**66**). The hydantoin was subject to base hydrolysis followed by debenzoylation to give [<sup>13</sup>C]- $\alpha$ -methyldopa hydrochloride (**67**), which was used in biotransformation studies. The 5,5-cyclic disubstituted hydantoin sorbinil, an aldose reductase inhibitor with potential utility in the treatment of diabetes, has been prepared with a <sup>14</sup>C label at C-4 in the hydantoin ring or with a deuterium or tritium atom at a single position on the aromatic ring of the substituent group (**70a**, **70b**, **70c**) (Figure 13, Scheme 21) [106]. The <sup>14</sup>C label was introduced by Bucherer-Bergs reaction with the appropriate ketone (**68**) and [<sup>14</sup>C]potassium cyanide; deuterium or tritium was introduced by reductive dehalogenation of the

chloride derivative (**69**) with deuterium or tritium gas. The pharmacologically active *S*-(+)-enantiomer was isolated by brucine resolution [100]. 5-Ethyl-5-phenylhydantoin has been synthesised with deuterium labelling at the methyl group (**71a**) or in the phenyl group (**71b**) or with a  $^{14}\text{C}$ -label at the C-4 position (**71c**) (Figure 13) during synthesis of the antimuscarinic drug trimebutine and its metabolites with deuterium and  $^{14}\text{C}$  labels [107]. The symmetric 5,5-cyclic disubstituted spirodihydantoin has been synthesised with  $^{13}\text{C}$  and  $^{15}\text{N}$  labels in one half of the molecule (**74a**) [108] using a method by Poje *et al* [109] (Figure 13, Scheme 22). Reaction of 4,5,6(1*H*)-pyrimidinetrione (**72**) with [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]urea (**26d**) and then bromine gave the 5,5-disubstituted hydantoin (**73**) which was cyclised to give [2- $^{13}\text{C}$ , 1,3- $^{15}\text{N}_2$ ]spirodihydantoin (**74a**). This compound was used to help demonstrate that spirodihydantoin is a minor product from oxidation of uric acid. A series of 5,5-disubstituted hydantoins with a sulphonamide as one of the substituent groups have been prepared with multiple  $^{13}\text{C}$  labels and with a  $^{15}\text{N}$  label at N-3 in the hydantoin ring (**81**) using simple compounds as sources of the labels (Figure 13, Scheme 23) [110]. [ $^{13}\text{C}_3$ ]Acetone (**75**) was converted into [ $^{13}\text{C}_3$ ]chloroacetone (**76**), which was alkylated with benzyl mercaptan (**77**) then the ketone (**78**) was subjected to a microwave-induced Bucherer-Bergs reaction with [ $^{13}\text{C}$ ,  $^{15}\text{N}$ ]potassium cyanide to give the 5,5-disubstituted hydantoin (**79**), which was converted to the sulphonyl chloride (**80**). This was coupled with a range of amines to give the hydantoin sulphonamide compounds (**81**) with  $^{13}\text{C}$  labels at C-4, C-5,  $\text{CH}_3$  and  $\text{CH}_2$  and  $^{15}\text{N}$  at N-3. The introduction of multiple labels provided a molecule with a greater mass differential from the parent compound that was more useful than a molecule with a single label in the development of bioanalytical methods.

## 6. N-SUBSTITUTED HYDANTOINS

Some hydantoins with substituents at the N-1 and/or N-3 position have been synthesised with isotope labels. The hydantoin drug dantrolene (**82**), which interferes with release of calcium ions from intracellular stores of skeletal muscle, and a photoaffinity azido analogue (**83**) have been synthesised with a tritium label at a non-exchangeable imine in the N-1 substituent group (Figure 14) [111]. The tritium label was introduced into [ $^3\text{H}$ ]dantrolene using sodium borotritide and into [ $^3\text{H}$ ]azidodantrolene using lithium triethylborotritide.  $^1\text{H}$  and  $^3\text{H}$  NMR analysis of the final tritiated compounds suggested  $\geq 95\%$   $^3\text{H}$  labelling since the spectra showed no sign of hydrogen at the imine position (Figure 15A). It is also reported that the compounds showed no sign of hydrogen/tritium exchange during a period of over one year [111]. The tritiated compounds were used in the identification of a putative skeletal muscle dantrolene binding site in sarcoplasmic reticulum membranes (Figure 15B) [111]. Dantrolene is also a substrate for breast cancer resistant protein (BCRP),

which is widely distributed in the blood-brain-barrier, intestine, gall bladder and liver.



**Figure 15** (Figures 13 and 14 are on pages 22 and 23). NMR analysis of [ $^3\text{H}$ ]dantrolene (**82**) and [ $^3\text{H}$ ]azidodantrolene (**83**) and use of [ $^3\text{H}$ ]azidodantrolene in identifying a dantrolene binding site in sarcoplasmic reticulum membranes. A. (i)  $^3\text{H}$  NMR spectrum of [ $^3\text{H}$ ]azidodantrolene in  $d_8$ -THF, (ii)  $^1\text{H}$  NMR spectrum of [ $^3\text{H}$ ]azidodantrolene in  $d_8$ -THF and (iii)  $^1\text{H}$  NMR spectrum of [ $^3\text{H}$ ]dantrolene in  $d_8$ -THF. B. Inhibition of [ $^3\text{H}$ ]azidodantrolene (200 nM) binding to sarcoplasmic reticulum membranes by increasing concentrations of dantrolene (triangles), azumolene (circles) or aminodantrolene (squares). Dantrolene and azumolene were equipotent whilst aminodantrolene was inactive. These figures were reproduced with permission from Palnitkar *et al* (1999) [111]; copyright © 1999 by the American Chemical Society.

Radiolabelled versions of dantrolene have been synthesised for use in positron emission tomography (PET) imaging studies that target BCRP. [2- $^{11}\text{C}$ -carbonyl]Dantrolene has been prepared in a multi-step/one-pot labelling sequence starting with ethyl 2-{2-[5-(4-nitrophenyl)furfurylidene]hydrazino} acetate and using [ $^{11}\text{C}$ ]phosgene as the labelling agent [112]. [ $^{13}\text{N}$ ]Dantrolene has been prepared using no-carrier-added [ $^{13}\text{N}$ ]ammonia as the labelling agent [113]. A number of hydantoin-substituted indole-2-carboxylic acid compounds

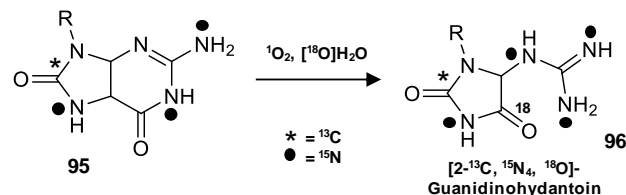


targeted at the *N*-methyl-*D*-aspartate (NMDA) receptor have been synthesised, which included two reference compounds with a fluoroethoxy or methoxy group labelled with  $^{18}\text{F}$  or  $^{11}\text{C}$  (**84**, **85**) (Figure 14), respectively, for PET imaging of the receptor glycine binding site [114]. These compounds containing a 1,3-di-*N*-substituted hydantoin moiety were obtained by alkylation of a phenolic hydroxyl group using [ $^{18}\text{F}$ ]-2-fluoroethyl tosylate or [ $^{11}\text{C}$ ]methyl iodide. A 2-nitrobenzaldehyde derivative of 1-aminohydantoin (**91**) has been synthesised with  $^{13}\text{C}$  labels in the aromatic ring starting from [1,2,3,4,5,6- $^{13}\text{C}_6$ ]toluene (**59b**) (Figure 14, Scheme 24) [115]. The [ $^{13}\text{C}_6$ ]toluene (**59b**) was nitrated using nitronium tetrafluoroborate giving a mixture of *ortho* and *para* products (**86**, **87**) which were then simultaneously oxidised using chromium trioxide to give a mixture of [1,2,3,4,5,6- $^{13}\text{C}_6$ ]-2-nitrobenzaldehyde (**88**) and [1,2,3,4,5,6- $^{13}\text{C}_6$ ]-4-nitrobenzaldehyde (**89**). Separation on a silica gel column using a step gradient of dichloromethane afforded the required *ortho* isomer in a yield of not more than 10% from [ $^{13}\text{C}_6$ ]toluene, which was then coupled with 1-aminohydantoin (**90**) to give (**91**). A full  $^1\text{H}$  and  $^{13}\text{C}$  NMR analysis of the final labelled compound was performed with chemical shift assignments assisted by [ $^1\text{H}$ - $^1\text{H}$ ]COSY, [ $^1\text{H}$ - $^{13}\text{C}$ ]HMQC and [ $^1\text{H}$ - $^{13}\text{C}$ ]HMBC 2D-correlation spectra and a homonuclear NOE difference spectrum exhibited a spatial correlation between the iminyl hydrogen and the hydrogen atoms N-CH $_2$  indicative of an *E* configuration [115]. This compound was used along with other  $^{13}\text{C}$ -labelled 2-nitrobenzaldehyde derivatives of nitrofurán metabolites as internal standards for the quantification of trace levels of nitrofurán residues by liquid chromatography-tandem mass spectrometry in foods of animal origin [115]. 3-Methyl-2-thiohydantoin (**93**) has been synthesised with a  $^{14}\text{C}$  label at C-4 in two steps from [1- $^{14}\text{C}$ ]glycine (**6d**) (Figure 14, Scheme 25) [116]. Reaction of the glycine with methyl isothiocyanate under basic conditions produced  $^{14}\text{C}$ -labelled 5-methylthiohydantoinic acid (**92**), which cyclises at acidic pH to [4- $^{14}\text{C}$ ]-3-methyl-2-thiohydantoin (**93**). This compound is a pharmacologically active metabolite of the antithyroid drug methimazole. A new antihypertensive agent 3-[4-4-(3-methylphenyl)-1-piperazinyl]butylhydantoin (**94**) was synthesised with two adjacent  $^{14}\text{C}$  labels in the piperazinyl ring in three steps from [ $^{14}\text{C}_2$ ]ethylene oxide with an overall radiochemical yield of 69% (Figure 14, Scheme 26) [117].

## 7. $^{18}\text{O}$ -LABELLED HYDANTOINS

The stable isotope oxygen-18 has been incorporated into some hydantoin compounds. Guanidinohydantoin has been synthesised with  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{18}\text{O}$  labels (**96**) by reaction of  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labelled guanosine (**95**) with singlet oxygen and [ $^{18}\text{O}$ ]water at a pH of less than 7 (Figure 16, Scheme 27) [118]. This isotope labelling of guanidinohydantoin combined with mass spectrometry and NMR analysis helped to resolve controversial issues concerning its structure and suggested potential mechanisms to explain

products observed from the oxidation of guanosine by singlet oxygen, which has importance in cancer etiology and its cure by photodynamic therapy. The same work that produced  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labelled spirodihydantoin (**74a**) described above also produced spirodihydantoin (**74b**), spiroiminohydantoin (**97**), allantoin (**2b**) and guanidinohydantoin (**96**) with an  $^{18}\text{O}$  label (Figure 16) [108]. These compounds were used in an investigation of uric acid metabolism.



Scheme 27

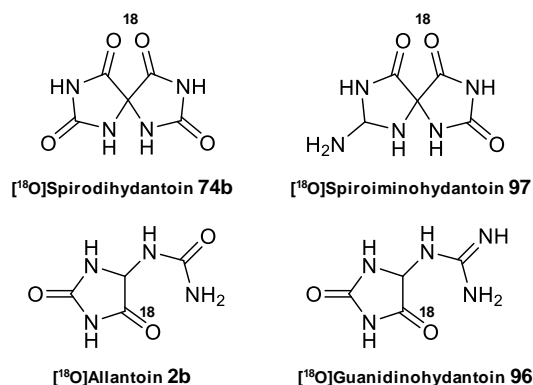
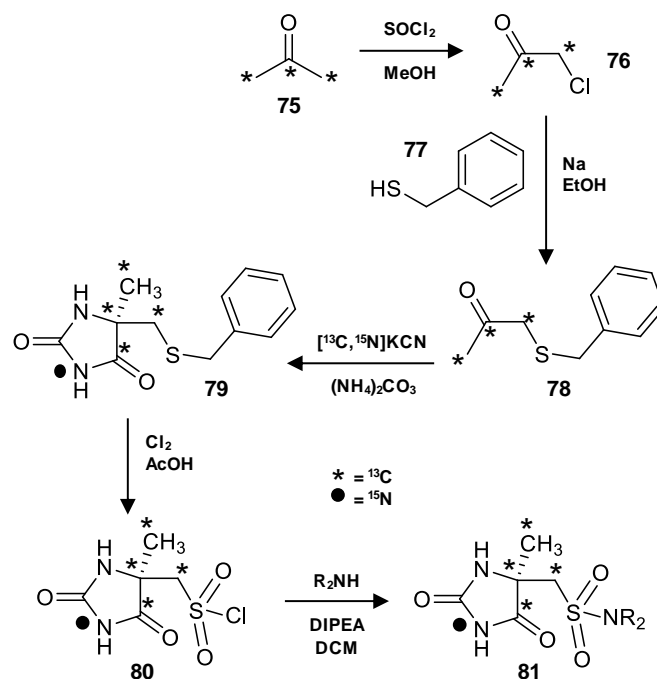
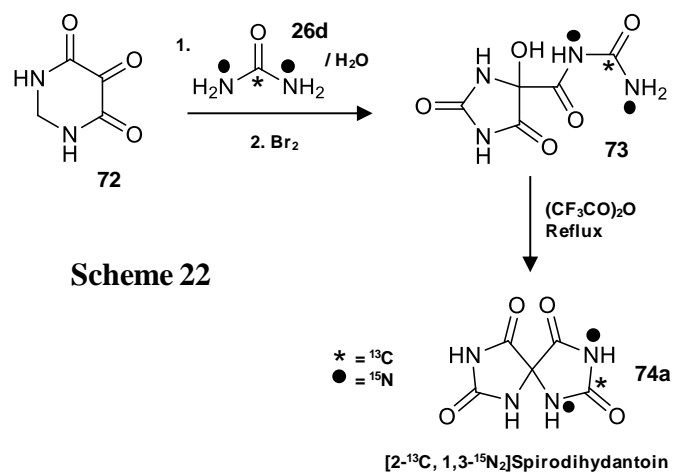
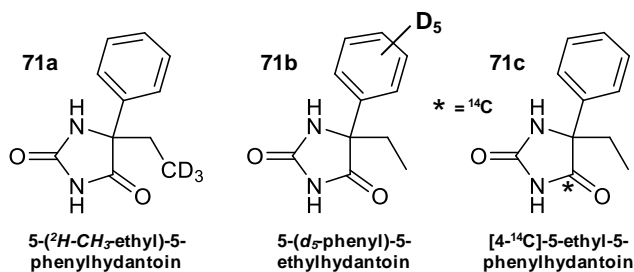
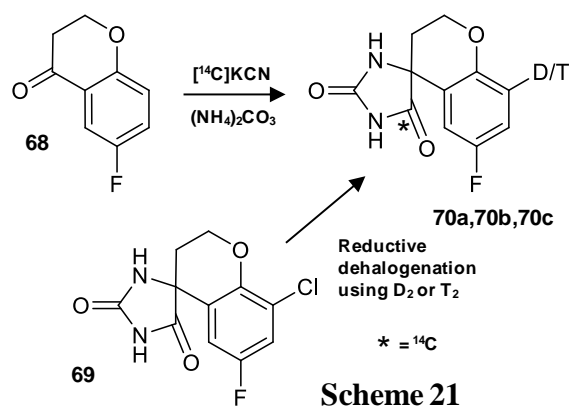
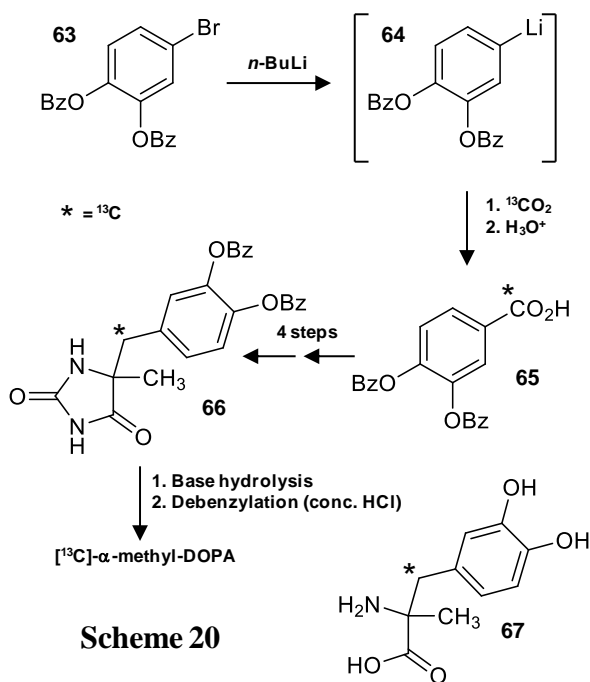


Figure 16.  $^{18}\text{O}$ -labelled hydantoin. Synthesis of guanidinohydantoin with  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{18}\text{O}$  labels (**96**) (Scheme 27) and structures of  $^{18}\text{O}$ -labelled spirodihydantoin (**74b**), spiroiminohydantoin (**97**), allantoin (**2b**) and guanidinohydantoin (**96**).

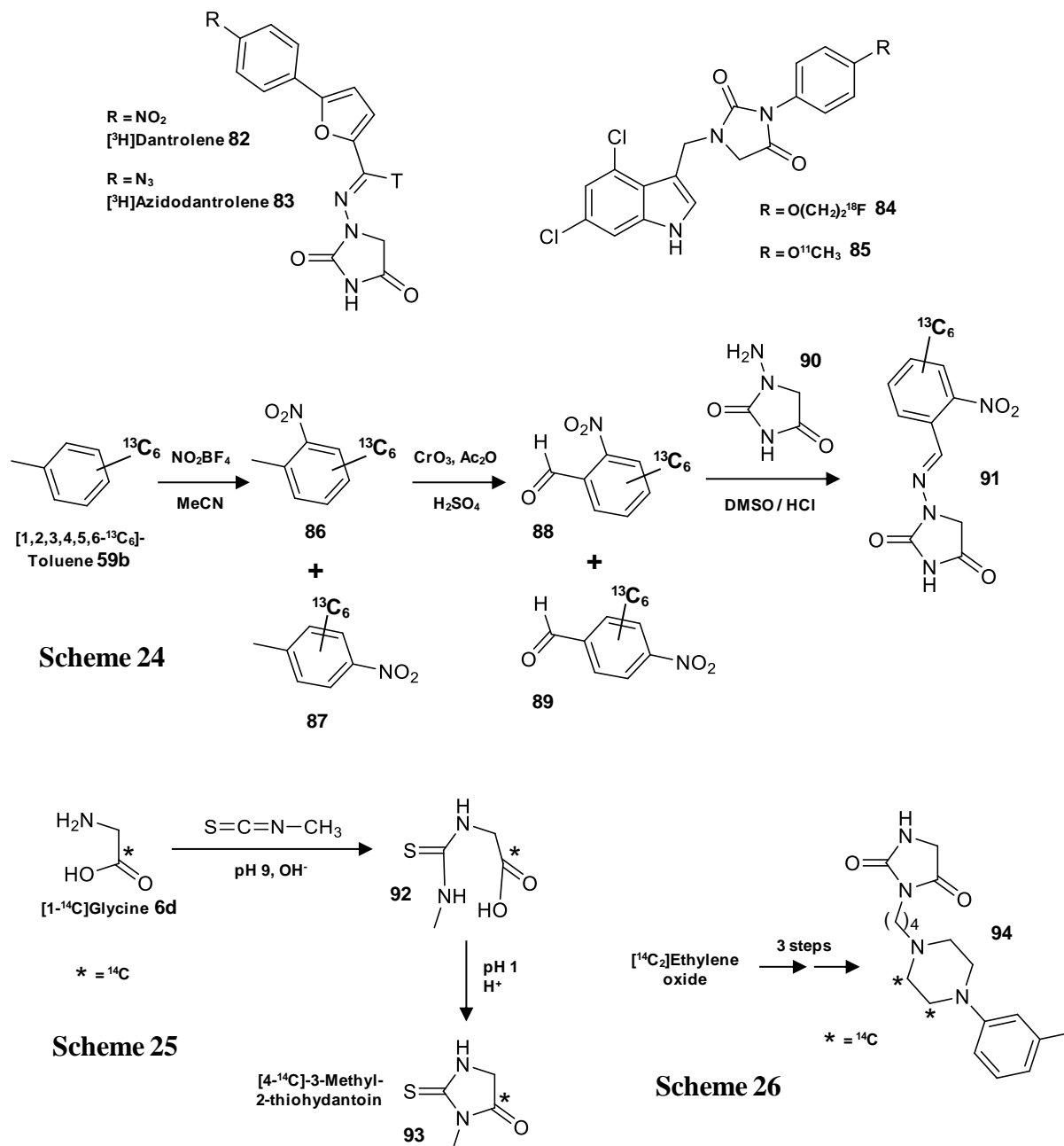
## 8. OTHER MORE COMPLEX MOLECULES THAT CONTAIN THE HYDANTOIN MOIETY

A range of compounds containing the hydantoin moiety with multiple and/or complex substituents have been synthesised containing isotope labels. The separate enantiomers of the anticonvulsant drug 3-methyl-5-ethyl-5-phenylhydantoin (mephentoin) (**98**) were synthesised with a  $^{14}\text{C}$  label at the 3-methyl group by reaction of the separate *R* and *S* forms of 5-ethyl-5-phenylhydantoin (**71**) with [ $^{14}\text{C}_2$ ]dimethylsulphate (Figure 17, Scheme 28) [119]. These compounds were used to measure their fate of metabolism in breath, blood and urine of the dog. The nitrogen mustard spiromustine (**101**) has been synthesised in an octadeuterated form (Figure 17, Scheme 29) [120]. *d* $_8$ -Ethylene glycol was converted *d* $_8$ -ethanolamine via a 2-imino-1,3-oxazolidine intermediate. The *d* $_8$ -ethanolamine was then reacted with 3-chloroethyl-spirohydantoin (**99**) to give *d* $_8$ -spiromustine (**101**) and other deuterated analogues. At the time, spiromustine was a potential new antitumor agent designed specifically for neoplasms of the central

nervous system and the deuterated form was required as an internal standard for selected ion monitoring by GC-MS.



**Figure 13. Synthesis of other 5,5-disubstituted hydantoin.** A  $^{13}\text{C}$ -labelled precursor to  $\alpha$ -methyl-DOPA (66) (**Scheme 20**), 5,5-cyclic disubstituted sorbinil with  $^{14}\text{C}$ , deuterium or tritium labels (70a, 70b, 70c) (**Scheme 21**), deuterated and  $^{14}\text{C}$ -labelled versions of 5-ethyl-5-phenylhydantoin (71a, 71b, 71c),  $[2-^{13}\text{C}, 1,3-^{15}\text{N}_2]$ spirodihydantoin (74a) (**Scheme 22**) and a series of  $^{13}\text{C}, ^{15}\text{N}$ -labelled hydantoin sulphonamide compounds (81) (**Scheme 23**).

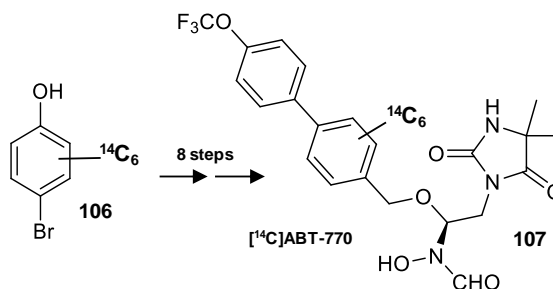
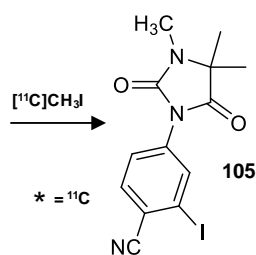
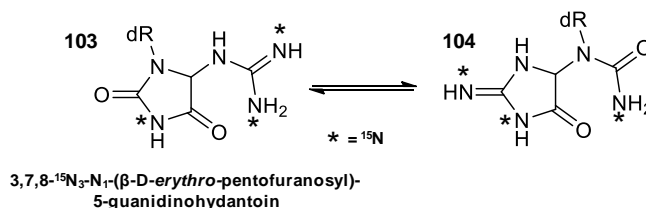
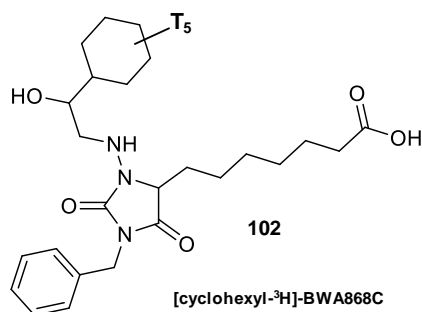
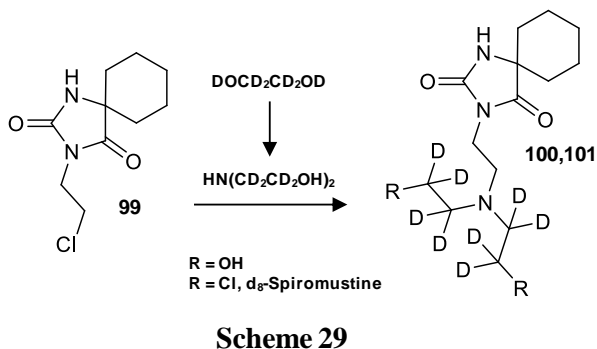
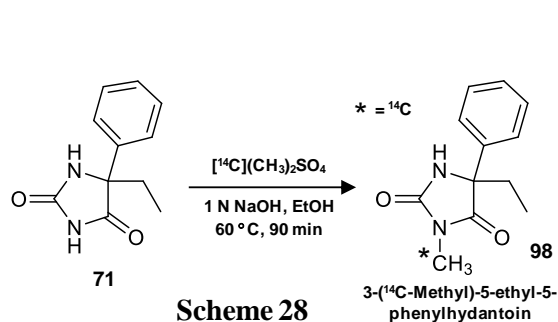


**Figure 14.** *N*-substituted hydantoin. Structures of [ $^3\text{H}$ ]dantrolene (82), [ $^3\text{H}$ ]azidodantrolene (83), and  $^{19}\text{F}$ - or  $^{11}\text{C}$ -labelled NMDA receptor compounds (84, 85), and syntheses of a  $^{13}\text{C}$ -labelled 2-nitrobenzaldehyde derivative of 1-aminohydantoin (91) (Scheme 24), [4- $^{14}\text{C}$ ]-3-methyl-2-thiohydantoin (93) (Scheme 25) and a  $^{14}\text{C}$ -labelled antihypertensive agent (94) (Scheme 26).

A DP prostanoid receptor antagonist BWA868C or 3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethyl-amino)hydantoin (102) has been synthesised with tritium labelling in the cyclohexyl group (Figure 17) and then used for characterisation and autoradiographic localisation of DP receptors in a range of mammalian tissues and cells and to better define the distribution and possible functions of DP receptors in the mammalian body [121]. This includes localisation of DP receptors in the

human eye (Figure 18) [121]. A  $^{15}\text{N}_3$ -labelled form of  $\text{N}^1$ -( $\beta$ -D-erythro-pentofuranosyl)-5-guanidinothiohydantoin (103) has been synthesised from [1- $^{15}\text{N}$ , 7- $^{15}\text{N}$ ,  $^{15}\text{NH}_2$ ]2'-deoxyguanosine for use as an internal standard in the measurement of  $\text{N}^1$ -( $\beta$ -D-erythro-pentofuranosyl)-5-guanidinothiohydantoin in peroxynitrite oxidized DNA by isotope-dilution mass spectrometry [122]. Compound 103 undergoes pH- and temperature-dependent isomerisation to

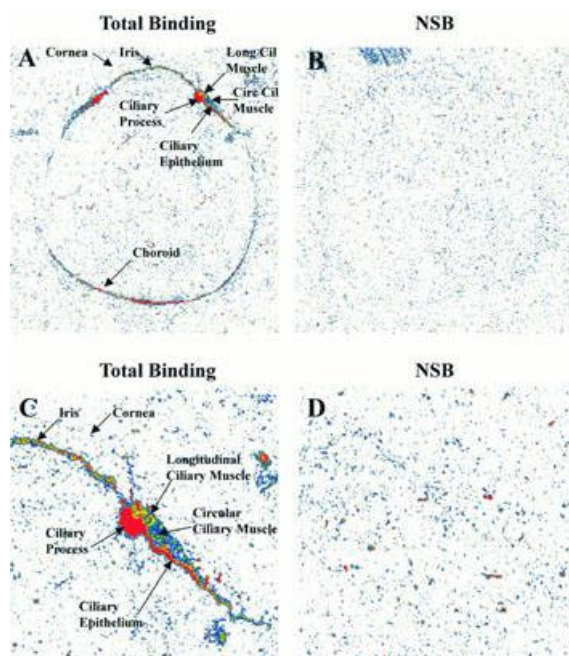
the iminoallantoin (**104**) (Figure 17), which made its purification especially challenging.



**Figure 17.** Syntheses of  $^{14}\text{C}$ -labelled mephenytoin (**98**) (Scheme 28) and  $d_8$ -spiromustine (**101**) (Scheme 29), structures of a tritium labelled DP prostanoid receptor antagonist BWA868C (**102**) and  $^{15}\text{N}_3$ -labelled N $^1$ -( $\beta$ -D-erythro-pentofuranosyl)-5-guanidinohydantoin (**103**) and syntheses of an  $^{11}\text{C}$ -labelled nonsteroidal androgen receptor ligand (**105**) (Scheme 30) and a  $^{14}\text{C}$ -labelled matrix metalloproteinase inhibitor ABT-770 (**107**) (Scheme 31).

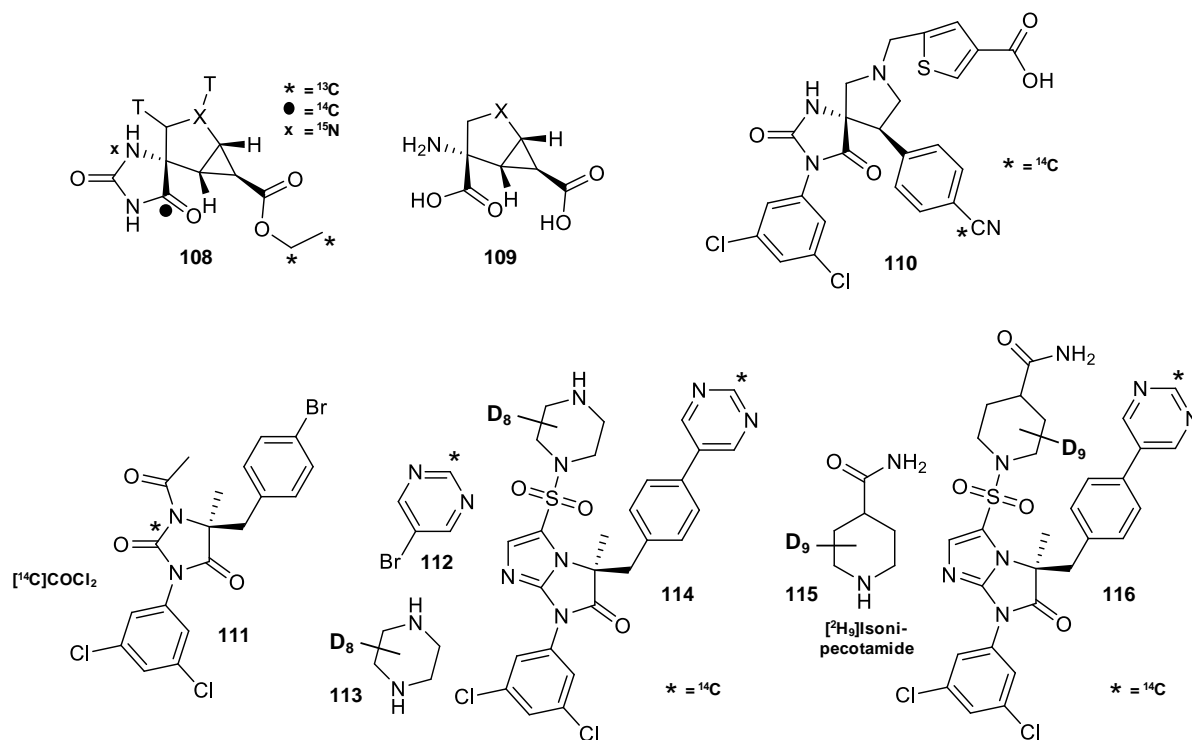
A multi-substituted hydantoin nonsteroidal androgen receptor ligand (**105**) was synthesised with an  $^{11}\text{C}$  label by introduction of the N-1 methyl group in the final step using [ $^{11}\text{C}$ ]methyl iodide (Figure 17, Scheme 30) [123]. This compound was used as a potential radioligand for PET imaging of prostate cancer. A matrix metalloproteinase inhibitor ABT-770: N-[(1S)-1-[(4,4-dimethyl-2,5-dioxo-1-imidazolidinyl)methyl]]-2-[[40-(trifluoromethoxy)[1,10-biphenyl]-4-yl]oxy]ethyl]-N-hydroxy formamide (**107**) was synthesised in 8 steps from [ $^{14}\text{C}_6$ ]4-bromophenol (**106**), which introduces  $^{14}\text{C}$ -labelling into a metabolically stable biphenyl ring (Figure 17, Scheme 31) [124]. The key part of the synthesis was a three-step one-pot reaction in which the hydantoin moiety was introduced, the imine oxidized and further hydrolysed to obtain the penultimate precursor to [ $^{14}\text{C}$ ]ABT-770 (**107**). The compound was used in animal

metabolism studies as a potential new antitumor agent. The synthesis of conformationally constrained analogues of glutamic acid: (+)-2-aminobicyclo[3.1.0]hexane-2,6-carboxylic acid and its 2-oxa and 2-thia-analogues with general structure **109** containing  $^3\text{H}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$  and  $^{15}\text{N}$  labels included a key hydantoin intermediate, for example **108**, which was formed by Bucherer-Burgs reaction with the appropriate chiral ketone and then hydrolysed to give **109** (Figure 19) [125]. Isotope labels were introduced using tritium gas, [ $^{13}\text{C}_2$ ]ethyl bromoacetate, [ $^{13}\text{C}$ ]potassium cyanide, [ $^{14}\text{C}$ ]potassium cyanide and [ $^{15}\text{N}$ ]ammonium chloride. This work also described the first use of *in situ* generated [ $^{15}\text{N}$ ]ammonium carbonate in the Bucherer-Bergs reaction, which was formed by reaction of [ $^{15}\text{N}$ ]ammonium chloride and sodium carbonate. These compounds had been identified as highly potent glutamate receptor agonists and



**Figure 18. Localisation of DP prostanoid receptors in the human eye using antagonist [ $^3\text{H}$ ]BWA868C.** Quantitative autoradiographs of DP prostanoid receptor binding sites (highest intensity = red) in sections of human eye using 5 nM [ $^3\text{H}$ ]BWA868C. Panels A and C show total binding, B and D show non-specific binding (NSB). Magnifications for the images are 3.4x (A and B) and 8.5x (C and D). This picture was reproduced with permission from Sharif et al (2000) [121]; copyright © 2000 by the British Pharmacological Society.

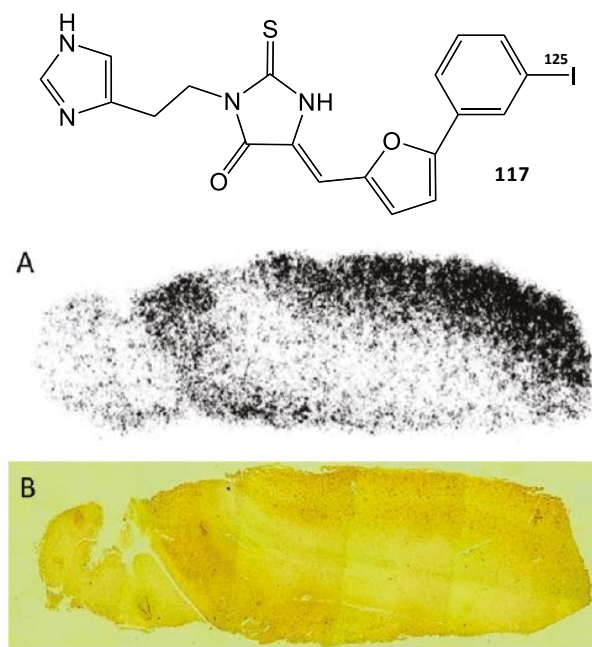
isotope-labelled versions were synthesised for preclinical ADME studies. The drug lead candidate leukocyte function-associated antigen 1 antagonist spirocyclic hydantoin: 5-(((5S,9R)-9-(4-cyanophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)methyl)-thiophene-3-carboxylic acid (**110**) (Figure 19) was synthesised with a  $^{14}\text{C}$  label at the cyano group by substitution of a bromide with [ $^{14}\text{C}$ ]zinc cyanide, prepared from zinc chloride and [ $^{14}\text{C}$ ]potassium cyanide, followed by two further radiochemical steps [126]. Three hydantoin based potent antagonists of lymphocyte function-associated antigen-1 labelled with  $^{14}\text{C}$  and deuterium were synthesised for drug metabolism and pharmacokinetics studies (Figure 19) [127]. (*R*)-1-acetyl-5-(4-bromobenzyl)-3-(3,5-dichlorophenyl)-5-methyl hydantoin (**111**) was prepared with a  $^{14}\text{C}$  label at C-2 in the hydantoin ring in two radiochemical steps using [ $^{14}\text{C}$ ]phosgene. (*R*)-5-(1-piperazinylsulfonyl)-1-(3,5-dichlorophenyl)-3-[4-(5-pyrimidinyl)benzyl]-3-methyl-1-*H*-imidazo[1,2-*a*]imidazol-2-one (**114**) and (*R*)-1-[7-(3,5-dichlorophenyl)-5-methyl-6-oxo-5-(4-pyrimidin-5-ylbenzyl)-6,7-dihydro-5H-imidazo[1,2-*a*]imidazole-3-sulfonyl]piperidin-4-carboxylic acid amide (**116**) were prepared with a  $^{14}\text{C}$  label in the pyrimidine ring using [2- $^{14}\text{C}$ ]-5-bromopyrimidine (**112**) in a Suzuki reaction with the appropriate boronic acid esters.



**Figure 19. Structures of a hydantoin intermediate with  $^3\text{H}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$  and  $^{15}\text{N}$  labels (**108**) used as a precursor to conformationally constrained analogues of glutamic acid and 2-oxa and 2-thia-analogues (**109**), a  $^{14}\text{C}$ -labelled spirocyclic hydantoin (**110**), and antagonists of lymphocyte function-associated antigen-1 with  $^{14}\text{C}$  and/or deuterium labels (**111**, **114**, **116**).**



A deuterated version of **114** was prepared by reaction of the sulphonyl chloride derivative with *ds*-piperazine (**113**) and a deuterated version of **116** was prepared by reaction of the sulphonyl chloride derivative with *d*<sub>9</sub>-isonipecotamide (**115**). A <sup>125</sup>I-labelled thiohydantoin derivative (**117**) (Figure 20) has been synthesised using [<sup>125</sup>I]sodium iodide as the source of the label in the last step via an iododestannylation reaction with hydrogen peroxide as the oxidant and used for detecting tau pathology in Alzheimer's disease [128]. *In vitro* experiments using tau and β-amyloid aggregates showed high specific binding of **117** to tau aggregates and in hippocampal sections of brains from Alzheimer's disease patients, **117** produced intense staining of neurofibrillary tangles (Figure 20). Furthermore, in experiments using normal mice, **117** showed good uptake into and rapid washout from the brain, suggesting that a <sup>123</sup>I-labelled version of **117** should be investigated as a PET radiotracer for imaging tau pathology [128].



**Figure 20.** Structure of a <sup>125</sup>I-labelled thiohydantoin derivative (**117**) and its use for detecting tau pathology in Alzheimer's disease. **A.** *In vitro* autoradiogram of a section of human Alzheimer's disease brain labelled with **117**. **B.** The same section of Alzheimer's disease brain immunostained with an antibody against hyperphosphorylated tau. The pictures in A and B were reproduced with permission from Ono *et al* (2011) [128]; copyright © 2011 by the American Chemical Society.

## 9. CONCLUSIONS

This review of the methods of synthesis, NMR analysis and applications of isotope-labelled hydantoins has confirmed the hydantoin ring system as an important centre in synthetic chemistry and as a component in a variety of different compounds including some with pharmacological activity. In some cases, NMR analysis of the labelled compounds was especially important for confirming purity, labelling integrity and molecule conformation. Hydantoin

compounds containing isotope labels have been used in a range of biological, biomedical, food and environmental applications including metabolic and *in vivo* tissue distribution studies, biochemical analysis of transport proteins, identification and tissue distribution of drug binding sites, drug metabolism and pharmacokinetic studies and as an imaging agent.

## CONFLICTS OF INTEREST

The author reports no conflicts of interest.

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