Stereocontrolled synthesis of deuterated phenylalanine derivatives through manipulation of an $N$-phthaloyl protecting group for the recall of stereochemistry. Application in the study of phenylalanine ammonia lyase

Christopher J. Easton,* Nicholas L. Fryer, James B. Kelly, and Katherine Kociuba

Research School of Chemistry, Institute of Advanced Studies, Australian National University, Canberra, ACT 0200, Australia
E-mail: easton@rsc.anu.edu.au

This paper is dedicated to Professor Don W. Cameron on the occasion of his retirement. (received 28 Jun 01; accepted 07 Aug 01; published on the web 15 Aug 01)

Abstract
The enantiomers of [2-$^2$H$_1$]phenylalanine and all four stereoisomers of [2,3-$^2$H$_2$]phenylalanine have been prepared from ($S$)-phenylalanine through the introduction of a chiral centre onto an $N$-phthaloyl protecting group for the recall of stereochemistry. Studies of the interaction of these labelled phenylalanines with ($S$)-phenylalanine ammonia lyase show that both the C-2 and C-3 hydrogens of the product trans-cinnamate undergo exchange with solvent in the presence of the enzyme. The mechanistic implications of this observation are discussed.

Keywords: Phenylalanine, deuterated phenylalanine derivatives, $N$-phthaloyl protecting group, stereochemistry, phenylalanine ammonia lyase

Introduction

($S$)-Phenylalanine ammonia lyase (PAL) is a plant enzyme which catalyses the elimination of ammonia and a proton from ($S$)-phenylalanine 1 (Scheme 1), to give trans-cinnamic acid 2 as required for the biosynthesis of lignins, flavanoids and coumarins.$^{1,2}$ The catalysis by PAL has been studied extensively and was thought to involve activation of the substrate through addition of its amino group to a dehydroalanine prosthetic group at the enzyme active site.$^3$ However, recent work indicates that the dehydroalanine is probably incorporated in a methyldiene imidazolone 3, and that electrophilic attack of this residue at the ortho-position of the substrate’s aromatic ring is more likely to be the mechanism of substrate activation.$^4$
Stereoselectively deuterated phenylalanine derivatives have been used to study the mechanism of catalysis by PAL. Battersby and co-workers examined the stereoselectivity of the proton transfer from the substrate. They observed that (2S,3R)-[3-2H1]phenylalanine underwent the enzyme catalysed reaction to give [3-2H1]-trans-cinnamic acid, while (2S,3S)-[3-2H1]phenylalanine gave the unlabelled acid, establishing that PAL removes the 3-pro-S hydrogen from (S)-phenylalanine in what is therefore formally an antiperiplanar elimination process. (R)-Phenylalanine is a competitive inhibitor of PAL and a poor substrate of the enzyme. We showed that (2R,3R)-[3-2H1]phenylalanine reacted with PAL to give [3-2H1]-trans-cinnamic acid containing 27% deuterium, while (2R,3S)-[3-2H1]phenylalanine gave the labelled acid with 92% retention of the deuterium. It follows that (R)-phenylalanine reacts with PAL by loss of ammonia and mainly the 3-pro-R hydrogen, in an antiperiplanar process analogous to that found for the (S)-enantiomer. However, the lack of specificity in the hydrogen removal from (R)-phenylalanine indicates that there is a competing minor reaction pathway, which most likely involves either isomerization to (S)-phenylalanine, before elimination, or a synperiplanar elimination. We now report our attempts to explore this putative isomerase activity, using α-deuterated and α,β-dideuterated phenylalanine derivatives, as well as the stereocontrolled synthesis of these substrates. The synthesis illustrates a new method for the preparation of chiral α-deuterated amino acids, which avoids an enzyme catalysed resolution or any need to separate enantiomers.
Results and Discussion

Chiral α-deuterated amino acids are usually prepared by treatment of the corresponding racemic unlabelled amino acids 6 with acetic anhydride and deuterium oxide, followed by resolution of the product labelled acetamides 7 with Hog Renal Acylase (Scheme 2). However, it is difficult to obtain the (R)-enantiomers (R)-8 pure using this method because that requires complete consumption of the (S)-isomers of the acetamides 7 by the enzyme. Instead, we decided to prepare both (S)- and (R)-[2-2H1]phenylalanine 13 and 16 from (S)-phenylalanine 1, through manipulation of an N-phthaloyl protecting group to recall stereochemistry (Scheme 3). This approach is based on the concept of self-reproduction or self-regeneration of chirality developed by Seebach et al., which we have exploited previously to prepare the individual enantiomers of 2,3-methanovaline.

Scheme 2

(S)-Phenylalanine 1 was treated with phthalic anhydride, and then with acidified methanol, to give the phenylalanine derivative 9. The reduction and solvolysis of this compound was carried out using the procedure of Speckamp et al. Accordingly, treatment with sodium borohydride in methanol at –10 °C for 15 min, followed by acidification and stirring at room temperature for 16 h, afforded a ca. 1:1 mixture of the methoxy amides 10 and 11. These diastereomers were separated by chromatography on silica and obtained in yields of 36 and 31%. The individual
methoxy amides 10 and 11 reacted with sodium methoxide in methanol-\(\text{H}^2\) at reflux for 4 h, to give mixtures of the deuterides 12 and 15, and 14 and 17, respectively. The components were separated from these mixtures, by chromatography on silica, and treated with acetic acid/hydrochloric acid to give the \(\alpha\)-deuterated phenylalanine derivatives 13 and 16, from 12 and 14, and 15 and 17, respectively. The deuterides 13 and 16 were obtained as single enantiomers (>95% ee) as determined by analysis of their \(N\)-acetylated methyl esters by gas chromatography, on a Chirasil–Val capillary column. Their mass and \(1\)H NMR spectra showed that they each contained ca. 95% deuterium, which was incorporated regiospecifically at the \(\alpha\)-position.

# The stereochemistry at this position has been assigned arbitrarily and may be the reverse, although compounds 10, 12 and 15 must have the same stereochemistry at the C3'-position, as must compounds 11, 14 and 17. The \(\alpha\)-hydroxy amide analogues of the methoxy amides 10 and 11 reacted with base by epimerisation at the C3'-position leading to each giving rise to racemic phenylalanine when elaborated.

\textbf{Scheme 3}
It was not necessary to assign the stereochemistry of the methoxy amides 10 and 11 at the 3-position of the isoindoline moiety in order to exploit the new chiral centre of these compounds, to distinguish and separate the stereoisomers of the deuterated derivatives 12, 14, 15 and 17 and assign their stereochemistry at the amino acid α-position. While the deuteration of 10 occurs with epimerisation at the α-carbon, the products 12 and 15 are separable diastereomers, with the one 12 having similar physical and spectral properties to those of the starting material 10, and therefore possessing α-(S)-stereochemistry. It follows that the other product 15 has α-(R)-stereochemistry and similar physical and spectral properties to those of the diastereomer 11 of the precursor 10, since 15 is the deuterated enantiomer of 10. A similar rationale applies for the reaction of 11. Removal of the protecting groups from the deuterated methoxy amides 12, 14, 15 and 17 occurs with retention of configuration at the α-carbon, so the pairs of diastereomers 12 and 14, and 15 and 17, afford 13 and 16, respectively.

Using a similar approach it was possible to prepare each of the four stereoisomers of α,β-dideuterated phenylalanine 25, 28, 33 and 36 in a stereocontrolled manner, beginning with the (S)-phenylalanine derivative 9 (Scheme 4). Previously we have reported the use of the N-phthaloyl protecting group for side chain halogenation of amino acid derivatives without loss of stereochemical integrity at the α-carbon. Accordingly, the phenylalanine derivative 9 reacted with N-bromosuccinimide to give the bromides 18 and 19, which underwent deuterolysis with retention of configuration on treatment with deuterium over palladium on carbon, to give 20 and 21, respectively. When the (2S,3S)-phenylalanine derivative 20 was elaborated, as shown in Scheme 3 for the non-deuterated analogue 9, 25 and 28 were obtained, while a similar series of reactions carried out using 21 as the starting material gave 33 and 36 (Scheme 5). The deuterides 25, 28, 33 and 36 were obtained as single enantiomers (>95% ee). They were diastereomerically pure within the limits of detection using 1H NMR spectroscopy. Their mass spectra showed that they were ca. 95% dideuterated.

The synthesis of the deuterated phenylalanines 16, 28 and 36 involves overall inversion of stereochemistry at the α-carbon. The methodology is general, as shown through the conversion of the (S)-isomers of alanine 38a, valine 38b and leucine 38c to the corresponding (R)-enantiomers 44a–c, as illustrated in Scheme 6.

With the deuterated phenylalanines 13, 16, 25, 28, 33 and 36 in hand, their reactions with PAL in 0.04 mol L⁻¹ sodium borate buffer (pH 8.7) at 30 °C were investigated. The samples of trans-cinnamic acid 2 isolated from these reactions contained various amounts of deuterium, decreasing as either the enzyme-substrate ratio or the incubation time increased, to the extent that unlabelled cinnamate 2 was obtained if sufficient enzyme and long incubation times were employed. When (S)-phenylalanine 1 was used as the substrate and the reaction was carried out in deuterium oxide, the initial product was unlabelled cinnamate 2, but after further incubation extensive deuterium incorporation occurred at both the α- and β-positions. No deuterium incorporation was observed in the absence of enzyme. These exchange processes do not alter the principle conclusions drawn from work with the β-deuterated phenylalanines 4a,b and 5a,b, that were based on the extent of deuterium retention in samples of the cinnamate 2 formed initially. It
is still reasonable to conclude that \((S)-\)phenylalanine 1 reacts via loss of the pro-\(S\) hydrogen, while \((R)-\)phenylalanine reacts mainly by loss of the pro-\(R\) hydrogen. However, the exchange of the C-2 and C-3 hydrogens of the cinnamate 2 with deuterated solvent, and *vice versa*, in the presence of the enzyme does make it impractical to use the deuterated phenylalanines 13, 16, 25, 28, 33 and 36 to examine minor reaction pathways or study subtle isotope effects. As to how the exchange processes occur, it seems likely that the cinnamate 2 bound in the active site of PAL can undergo reversible nucleophilic addition at either the \(\alpha\)- or \(\beta\)-position. Reaction at the \(\beta\)-position is the normal pathway for conjugate addition, reflecting the polarisation of the cinnamate 2. The alternative pathway is probably facilitated by PAL through reaction of the methylidene imidazolone 3 with the aromatic ring of the cinnamate 2 (Figure 1).

# The stereochemistry at this position has been assigned arbitrarily and may be the reverse, although compounds 22, 24 and 27 must have the same stereochemistry at the C3'-position, as must compounds 23, 26 and 29.

**Scheme 4**
The stereochemistry at this position has been assigned arbitrarily and may be the reverse, although compounds 30, 32 and 35 must have the same stereochemistry at the C3'-position, as must compounds 31, 34 and 37.

Scheme 5
The stereochemistry at this position has been assigned arbitrarily and may be the reverse, although compounds 40 and 42 must have the same stereochemistry at the C3'-position, as must compounds 41 and 43.

Scheme 6

Figure 1. Activation of the cinnamate 2 by PAL to nucleophilic addition at the α-position.

Experimental Section

General Procedures. $^1$H NMR (300 MHz) spectra were recorded on a GEMINI 300 spectrometer and refer to deuterochloroform solutions with chloroform as the internal standard measured at δ 7.26 ppm, unless otherwise stated. Electron impact (EI) mass spectra were recorded on an AEI MS-30 spectrometer operating at 70 eV. Microanalyses were carried out by the Microanalytical Laboratory of the Research School of Chemistry at the Australian National
University. HPLC was performed using a Waters µ-Porasil silica column (5 µm silica, 19 • 300 mm), eluting with hexanes-ethyl acetate (5:1). GC was performed using a Chirasil–Val capillary column (0.3 mm • 25 m) and argon as the carrier gas with a flow rate of 0.5 mL min⁻¹.

**Materials.** (S)-Phenylalanine 1, (S)-alanine 38a, (S)-valine 38b and (S)-leucine 38c and the corresponding (R)-enantiomers and racemic materials were purchased from Sigma Chem. Co. and used to prepare the esters 9 and 39a–c using standard methods. Reaction of the ester 9 with N-bromosuccinimide to give the bromides 18 and 19, and their conversion to the corresponding deuterides 20 and 21, was carried out as previously reported. PAL (grade I from *Rhodotorula glutinis*) was obtained as a solution in 60% glycerol, 3 • 10⁻³ mol L⁻¹ tris-hydrochloric acid, pH 7.5, with an activity of ca. 3 • 10³ units L⁻¹.

**General procedure for reduction and solvolysis of the phthalimides 9, 20, 21 and 39a–c**

Sodium borohydride (190 mg, 5 mmol) was added slowly to a solution of the phthalimide (4.5 mmol) in dry methanol (50 mL), maintained at –10 °C. The mixture was stirred at that temperature for 15 min, then it was acidified through the cautious addition of thionyl chloride (1.0 g, 8.5 mmol). The resultant solution was allowed to warm to room temperature, then it was stirred for 16 h at room temperature, before it was poured into dilute aqueous ammonium chloride (50 mL). This solution was extracted with dichloromethane and the extract was dried and concentrated under reduced pressure. Analysis of the residual oil using ¹H NMR spectroscopy and HPLC showed that the corresponding α-methoxy amides were produced in a ca. 1:1 ratio. They were separated through chromatography on silica, eluting with hexanes–ethyl acetate.

Methyl (2S,3'R)-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenyl-propanoate (10) and methyl (2S,3'S)-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate (11). Reaction of (S)-N-phthaloylphenylalanine methyl ester 9 (1.39 g) afforded the title compounds 10 (527 mg, 36%) (Found: C, 69.94; H, 5.86; N, 4.32. C₁₉H₁₉NO₄ requires C, 70.14; H, 5.89; N, 4.30%); HPLC Rₜ 12.5 min; ¹H NMR δ 2.85 (3H, s), 3.50 (2H, m), 3.78 (3H, s), 4.64 (1H, dd, J = 6.5 and 9.5 Hz), 5.10 (1H, s), 7.1–7.9 (9H, m); MS m/z: 325 (M⁺, 18%), 293 (48), 266 (58), 132 (100) and 11 (460 mg, 31%) (Found: C, 70.20; H, 5.69; N, 4.27. C₁₉H₁₉NO₄ requires C, 70.14; H, 5.89; N, 4.30%); HPLC Rₜ 13.5 min; δ 2.39 (3H, s), 3.59 (1H, dd, J = 6 and 14.5 Hz), 3.71 (dd, J = 11 and 14.5 Hz), 3.75 (3H, s), 4.76 (1H, dd, J = 6 and 11 Hz), 5.80 (1H, s), 7.1–7.9 (9H, m); MS m/z: 325 (M⁺, 22%), 293 (43), 266 (58), 132 (100).

Methyl (2S,3'S,3'R)-[3-²H₁]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenyl-propanoate (22) and methyl (2S,3'S,3'R)-[3-²H₁]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate (23). Reaction of (2S,3S)-[3-²H₁]-N-phthaloylphenylalanine methyl ester 20 afforded the title compounds 22; HPLC Rₜ 12.5 min; ¹H NMR δ 2.84 (3H, s), 3.51 (1H, d, J = 9.5 Hz), 3.78 (3H, s), 4.62 (1H, d, J = 9.5 Hz), 5.11 (1H, s), 7.1–7.9 (9H, m); MS m/z: 326 (M⁺, 98% ²H₁); and 23; HPLC Rₜ 13.5 min; ¹H NMR δ 2.39 (3H, s), 3.70 (1H, d, J = 11 Hz), 3.75 (3H, s), 4.76 (1H, d, J = 11 Hz), 5.81 (1H, s), 7.1–7.9 (9H, m); MS m/z: 326 (M⁺, 99% ²H₁).
The deuterides 22 and 23 were stereochemically pure, within the limits of detection using $^1$H NMR spectroscopy (>95%). Their other properties were comparable with those of the unlabelled analogues 10 and 11.

**Methyl (2S,3R,3'R)-[3-$^2$H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate (30) and methyl (2S,3R,3'S)-[3-$^2$H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate (31).** Reaction of (2S,3R)-[3-$^2$H1]-N-phthaloylphenylalanine methyl ester 21 afforded the title compounds 30; HPLC $R_t$ 12.5 min; $^1$H NMR $\delta$ 2.84 (3H, s), 3.50 (1H, d, $J = 6.5$ Hz), 3.76 (3H, s), 4.63 (1H, d, $J = 6.5$ Hz), 5.10 (1H, s), 7.1–7.9 (9H, m); MS $m/z$: 326 (M+, 98% $^2$H1); and 31; HPLC $R_t$ 13.5 min; $^1$H NMR $\delta$ 2.39 (3H, s), 3.59 (1H, d, $J = 6$ Hz), 3.76 (3H, s), 4.75 (1H, d, $J = 6$ Hz), 5.81 (1H, s), 7.1–7.9 (9H, m); MS $m/z$: 326 (M+, 99% $^2$H1). The deuterides 30 and 31 were stereochemically pure, within the limits of detection using $^1$H NMR spectroscopy (>95%). Their other properties were comparable with those of the unlabelled analogues 10 and 11.

**Methyl (2S,3'R)-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-propanoate (40a) and methyl (2S,3'S)-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-propanoate (41a).** Reaction of (S)-N-phthaloylalanine methyl ester 39a afforded the title compounds 40a (476 mg, 42%); HPLC $R_t$ 4.5 min; $^1$H NMR $\delta$ 1.65 (3H, d, $J = 7.5$ Hz), 2.95 (3H, s), 3.74 (3H, s), 4.89 (1H, q, $J = 7.5$ Hz), 6.12 (1H, s), 7.5–7.9 (4H, m); MS $m/z$: 249 (M+, 15%), 217 (18), 190 (37), 132 (100); and 41a (428 mg, 38%); HPLC $R_t$ 5 min; $^1$H NMR $\delta$ 1.69 (3H, d, $J = 7$ Hz), 2.99 (3H, s), 3.74 (3H, s), 4.67 (1H, q, $J = 7$ Hz), 5.96 (1H, s), 7.5–7.9 (4H, m); MS $m/z$: 249 (M+, 38%), 217 (42), 190 (69), 132 (100).

**Methyl (2S,3'R)-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-methyl-butanoate (40b) and methyl (2S,3'S)-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-methylbutanoate (41b).** Reaction of (S)-N-phthaloylvaline methyl ester 39b afforded the title compounds 40b (445 mg, 36%); HPLC $R_t$ 6 min; $^1$H NMR $\delta$ 0.84 (3H, d, $J = 7$ Hz), 1.10 (3H, d, $J = 7$ Hz), 2.62 (1H, m), 2.91 (3H, s), 3.72 (3H, s), 4.49 (1H, d, $J = 10$ Hz), 5.90 (1H, s), 7.5–7.9 (4H, m); MS $m/z$: 277 (M+, 8%), 245 (6), 218 (14), 132 (100); and 41b (379 mg, 30%); HPLC $R_t$ 6.5 min; $^1$H NMR $\delta$ 0.96 (3H, d, $J = 7$ Hz), 1.22 (3H, d, $J = 7$ Hz), 2.08 (1H, m), 2.99 (3H, s), 3.73 (3H, s), 4.80 (1H, dd, $J = 6$ and 10 Hz), 6.14 (1H, s), 7.5–7.9 (4H, m); MS $m/z$: 291 (M+, 19%), 259 (22), 232 (46), 132 (100); and 41c (490 mg, 37%); HPLC $R_t$ 12 min; $^1$H NMR $\delta$ 0.95 (3H, d, $J = 7$ Hz), 0.98 (3H, d, $J = 7$ Hz), 1.53 (1H, m), 1.97 (1H, m), 2.11 (1H, m), 2.97 (3H, s), 3.72 (3H, s), 4.79 (1H, dd, $J = 6$ and 10 Hz), 5.88 (1H, s), 7.5–7.9 (4H, m); MS $m/z$: 291 (M+, 45%), 259 (52), 232 (78), 132 (100).
General procedure for deuteration of the α-methoxy amides 10, 11, 22, 23, 30 and 31. A solution prepared from sodium (48 mg, 2 mmol) and methanol-\(\text{D}_2\) (1 mL) was added to a solution of the α-methoxy amide (1 mmol) in methanol-\(\text{D}_2\) (20 mL), and the mixture was heated at reflux for 4 h, then it was poured cautiously into dilute aqueous hydrochloric acid (50 mL). The resultant solution was extracted with dichloromethane and the extract was dried and concentrated under reduced pressure. Analysis of the residual oil using \(^1\)H NMR spectroscopy and HPLC showed that the corresponding deuterated α-methoxy amides were produced in a ca. 1:1 ratio. They were separated through chromatography on silica, eluting with hexanes–ethyl acetate.

Methyl \((2S,3'S')-\text{[2-}^2\text{H}1\text{]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(12\text{) and methyl \((2R,3'R')-\text{[2-}^2\text{H}1\text{-}\text{2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(15\). Reaction of methyl \((2S,3'S')-\text{[3-}^2\text{H}1\text{-}2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(10\text{ affor}}

Methyl \((2S,3'S)-\text{[2-}^2\text{H}1\text{]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(14\) and methyl \((2R,3'S')-\text{[2-}^2\text{H}1\text{-}\text{2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(17\). Reaction of methyl \((2S,3'S)-\text{[2,3-}^2\text{H}2\text{-}2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(11\text{ affor}}

Methyl \((2S,3'S')-\text{[2,3-}^2\text{H}2\text{-}2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(24\) and methyl \((2R,3'S,3'R')-\text{[2,3-}^2\text{H}2\text{-}2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(27\). Reaction of methyl \((2S,3'S,3'R')-\text{[3-}^2\text{H}1\text{-}\text{2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate \(22\text{ affor}}
isoindol-2-yl)-3-phenylpropanoate (29). Reaction of methyl (2S,3S,3'S)-[3-2H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate 23 afforded the title compounds 26; HPLC Rt 13.5 min; 1H NMR δ 2.38 (3H, s), 3.69 (1H, s), 3.76 (3H, s), 5.82 (1H, s), 7.1–7.9 (9H, m); MS m/z: 327 and 326 (M+, 94% 2H2, 98% 2H1); and 29; HPLC Rt 12.5 min; 1H NMR δ 2.83 (3H, s), 3.50 (1H, s), 3.77 (3H, s), 5.10 (1H, s), 7.1–7.9 (9H, m); MS m/z: 327 and 326 (M+, 94% 2H2, 99% 2H1). The deuterides 26 and 29 were stereochemically pure, within the limits of detection using 1H NMR spectroscopy (>95%). Their other properties were comparable with those of the unlabelled analogue 11 and the unlabelled enantiomer 10.

Methyl (2S,3R,3'R)-[2,3-2H2]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate (32) and methyl (2R,3R,3'R)-[2,3-2H2]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate (35). Reaction of methyl (2S,3R,3'R)-[3-2H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate 30 afforded the title compounds 32; HPLC Rt 12.5 min; 1H NMR δ 2.83 (3H, s), 3.51 (1H, s), 3.75 (3H, s), 5.11 (1H, s), 7.1–7.9 (9H, m); MS m/z: 327 and 326 (M+, 95% 2H2, 98% 2H1); and 35; HPLC Rt 13.5 min; 1H NMR δ 2.40 (3H, s), 3.60 (1H, s), 3.77 (3H, s), 5.80 (1H, s), 7.1–7.9 (9H, m); MS m/z: 327 and 326 (M+, 93% 2H2, 99% 2H1). The deuterides 32 and 35 were stereochemically pure, within the limits of detection using 1H NMR spectroscopy (>95%). Their other properties were comparable with those of the unlabelled analogue 10 and the unlabelled enantiomer 11.

Methyl (2S,3R,3'S)-[2,3-2H2]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate (34) and methyl (2R,3R,3'S)-[2,3-2H2]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate (37). Reaction of methyl (2S,3R,3'S)-[3-2H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1H-isoindol-2-yl)-3-phenylpropanoate 31 afforded the title compounds 34; HPLC Rt 13.5 min; 1H NMR δ 2.39 (3H, s), 3.58 (1H, s), 3.77 (3H, s), 5.80 (1H, s), 7.1–7.9 (9H, m); MS m/z: 327 and 326 (M+, 94% 2H2, 99% 2H1); and 37; HPLC Rt 12.5 min; 1H NMR δ 2.85 (3H, s), 3.49 (1H, s), 3.77 (3H, s), 5.11 (1H, s), 7.1–7.9 (9H, m); MS m/z: 327 and 326 (M+, 94% 2H2, 98% 2H1). The deuterides 34 and 37 were stereochemically pure, within the limits of detection using 1H NMR spectroscopy (>95%). Their other properties were comparable with those of the unlabelled analogue 11 and the unlabelled enantiomer 10.

General procedure for epimerisation of the α-methoxy amides 40a–c and 41a–c

A solution prepared from sodium (48 mg, 2 mmol) and methanol (1 mL) was added to a solution of the α-methoxy amide (1 mmol) in methanol (20 mL), and the mixture was heated at reflux for 4 h, then it was poured cautiously into dilute aqueous hydrochloric acid (50 mL). The resultant solution was extracted with dichloromethane and the extract was dried and concentrated under reduced pressure. Analysis of the residual oil using 1H NMR spectroscopy and HPLC showed that the starting material and the corresponding isomeric α-methoxy amide were present in a ca. 1:1 ratio. They were separated through chromatography on silica, eluting with hexanes-ethyl acetate.

The α-methoxy amides 42a–c and 43a–c prepared in this manner were isolated in yields ranging from 32–43%, and had properties comparable with those of their corresponding enantiomers.
41a–c and 40a–c.

General procedure for deprotection of the amino acid derivatives 12, 14, 15, 17, 24, 26, 27, 29, 32, 34, 35, 37, 42a–c and 43a–c. A solution of the amino acid derivative (0.5 mmol) in a 2:1 mixture of 6N hydrochloric acid and acetic acid (20 mL) was heated at reflux for 5 h and stirred at room temperature for 16 h, before being concentrated under reduced pressure. Water was added to the residue and the mixture was filtered. The filtrate was concentrated under reduced pressure and this residue was dissolved in a mixture of ethanol (10 mL), aniline (0.7 mL) and dichloromethane (10 mL). The solution was allowed to stand at 4 °C for 24 h, and the crystals which formed were separated by filtration and washed with dichloromethane, to give the corresponding free amino acid.

The amino acids 13, 16, 25, 28, 33, 36 and 44a–c prepared in this manner from 12 and 14, 15 and 17, 24 and 26, 27 and 29, 32 and 34, 35 and 37, and 42a–c and 43a–c, respectively, were isolated in yields ranging from 78–91%. The samples of (R)-alanine (44a), (R)-valine (44b) and (R)-leucine (44c) were identical with authentic specimens. The samples of the labelled phenylalanine derivatives 13, 16, 25, 28, 33 and 36 had properties comparable with those of unlabelled (S)-phenylalanine 1 and the (R)-enantiomer. Each of the amino acids 13, 16, 25, 28, 33, 36 and 44a–c was shown to be a single enantiomer (>95% ee) using the following procedure. Treatment of a small sample (ca. 1 mg) with acetic anhydride (5 mg) and triethylamine (10 mg) in water (1 mL) for 2 h at room temperature, followed by acidification and extraction with ethyl acetate, gave the corresponding acetamide. This was added to methanol (1 mL) which had been pretreated with thionyl chloride (10 mg), and the mixture was stirred at room temperature for 2 h, then concentrated under reduced pressure, to give the crude N-acetylated amino acid methyl ester, which was analysed by GC and compared with racemic material. The dideuterides 25, 28, 33 and 36 were diastereochmically pure, within the limits of detection using 1H NMR spectroscopy (>95%).

(2S)-[2-2H1]-Phenylalanine (13). Prepared from 12 and 14, the title compound 13 had 1H NMR δ (2H2O) 3.03 (1H, d, J = 14.5 Hz), 3.20 (1H, d, J = 14.5 Hz), 7.2–7.4 (5H, m); MS m/z: 167 (M+ + 1, 95% 2H1).

(2R)-[2-2H1]-Phenylalanine (16). Prepared from 15 and 17, the title compound 16 had 1H NMR δ (2H2O) 3.03 (1H, d, J = 14.5 Hz), 3.19 (1H, d, J = 14.5 Hz), 7.2–7.4 (5H, m); MS m/z: 167 (M+ + 1, 94% 2H1).

(2S,3S)-[2,3-2H2]-Phenylalanine (25). Prepared from 24 and 26, the title compound 25 had 1H NMR δ (2H2O) 3.02 (1H, s), 7.2–7.4 (5H, m); MS m/z: 168 and 167 (M+ + 1, 94% 2H2, 98% 2H1).

(2R,3S)-[2,3-2H2]-Phenylalanine (28). Prepared from 27 and 29, the title compound 28 had 1H NMR δ (2H2O) 3.20 (1H, s), 7.2–7.4 (5H, m); MS m/z: 168 and 167 (M+ + 1, 93% 2H2, 99% 2H1).

(2S,3R)-[2,3-2H2]-Phenylalanine (33). Prepared from 32 and 34, the title compound 33 had 1H NMR δ (2H2O) 3.21 (1H, s), 7.2–7.4 (5H, m); MS m/z: 168 and 167 (M+ + 1, 95% 2H2, 98% 2H1).
(2R,3R)-[2,3-2H2]-Phenylalanine (36). Prepared from 35 and 37, the title compound 36 had 1H NMR δ (2H2O) 3.03 (1H, s), 7.2–7.4 (5H, m); MS m/z: 168 and 167 (M+ + 1, 93% 2H2, 99% 2H1).

Reaction of (S)-phenylalanine 1 with PAL. Incubation of (S)-phenylalanine 1 (44 mg) with PAL (0.2 mL) in deuterated sodium borate buffer (0.04 mol L–1, pD 8.7), at 30 C for 24 h, afforded, after acidification and extraction with ethyl acetate, trans-cinnamic acid 2; 1H NMR δ 6.47 (1H, d, J = 16 Hz), 7.4–7.6 (5H, m), 7.81 (1H, d, J = 16 Hz); MS m/z: 148 (M+, 91%), 147 (100). When the reaction was repeated using only 1 mg of the substrate 2 and the mixture was left to incubate for 7 days, the product was deuterated trans-cinnamic acid 2 which had: 1H NMR δ 6.46 (0.2H, m), 7.4–7.6 (5H, m), 7.80 (0.1H, m); MS m/z: 150 and 149 (M+, 73% 2H2, 95% 2H1).

References