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

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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)

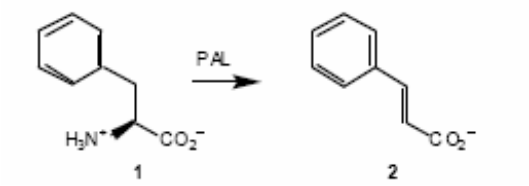
Abstract

The enantiomers of [2-$^2H_1$]phenylalanine and all four stereoisomers of [2,3-$^2H_2$]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.

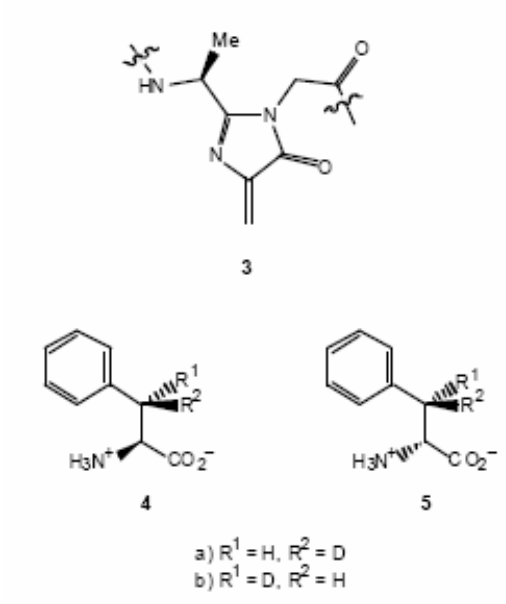
Keywords: Phenylalanine, deuterated phenylalanine derivatives, *N*-phthaloyl protecting group, stereochemistry, phenylalanine ammonia lyase

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 methylidene 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]



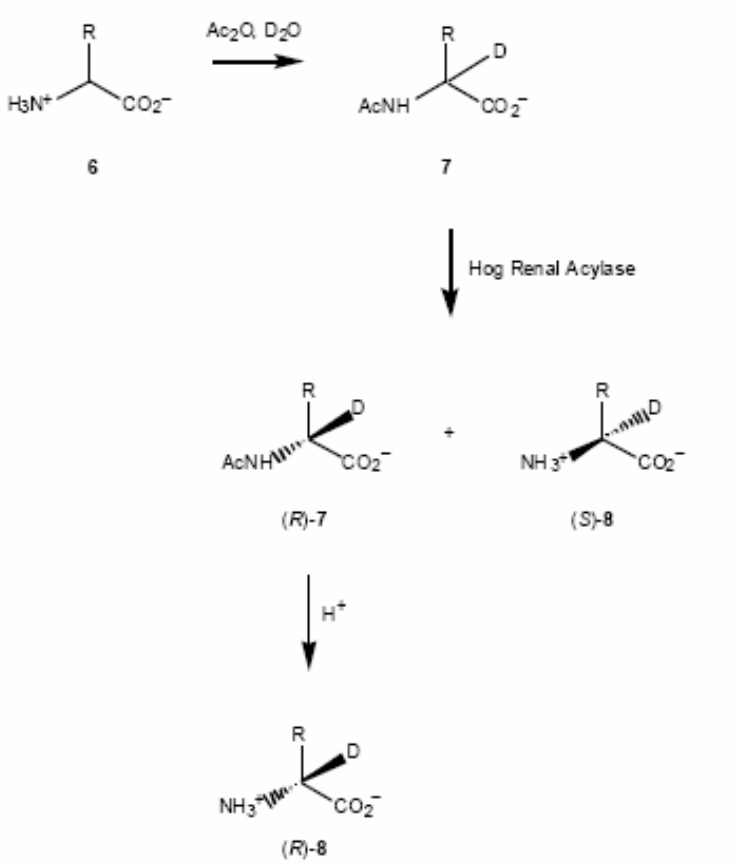
Scheme 1



Stereoselectively deuterated phenylalanine derivatives have been used to study the mechanism of catalysis by PAL.[5–7] Battersby and co-workers[5] examined the stereoselectivity of the proton transfer from the substrate. They observed that (2*S*,3*R*)-[3-$^2H_1$]phenylalanine **4a** underwent the enzyme catalysed reaction to give [3-$^2H_1$]-*trans*-cinnamic acid, while (2*S*,3*S*)-[3-$^2H_1$]phenylalanine **4b** 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.[8] We[6] showed that (2*R*,3*R*)-[3-$^2H_1$]phenylalanine **5a** reacted with PAL to give [3-$^2H_1$]-*trans*-cinnamic acid containing 27% deuterium, while (2*R*,3*S*)-[3-$^2H_1$]phenylalanine **5b** 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 **1**. 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 **1**, 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

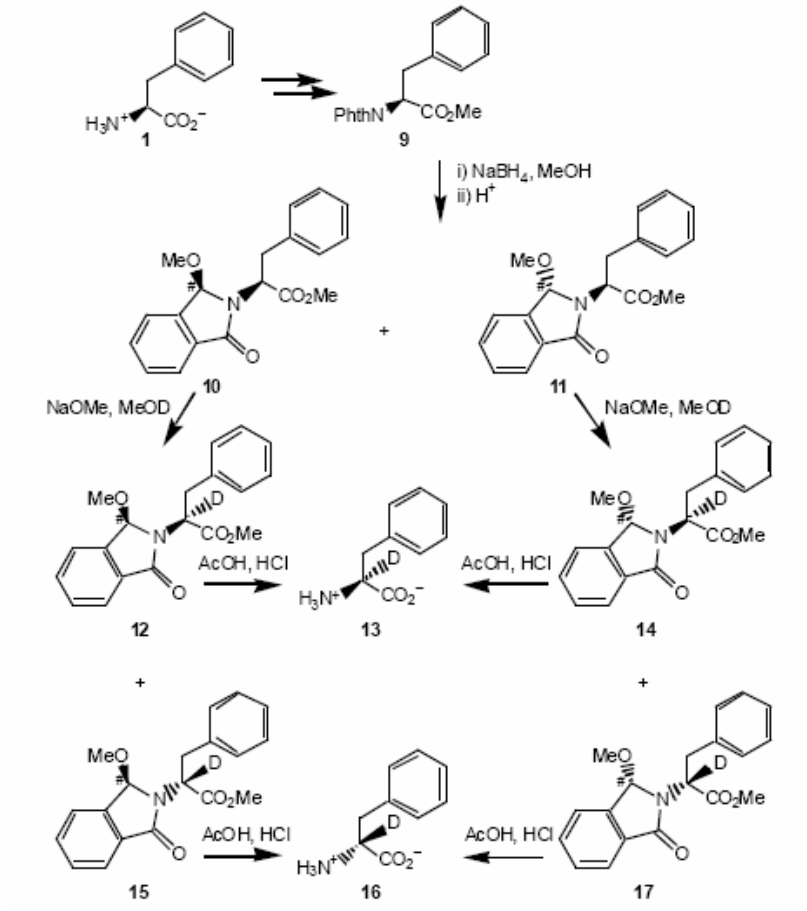
Chiral α-deuterated amino acids are usually prepared by treatment of the corresponding racemic unlabelled amino acids **6** with acetic anhydride and deuterium oxide, followed by resolution of the product labelled acetamides **7** with Hog Renal Acylase (Scheme 2).[9] However, it is difficult to obtain 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-$^{2}H_{1}$]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*.,[10] which we have exploited previously to prepare the individual enantiomers of 2,3-methanovaline.[11]



Scheme 2

(*S*)-Phenylalanine **1** was treated with phthalic anhydride, and then with acidified methanol, to give the phenylalanine derivative **9**.[12] The reduction and solvolysis of this compound was carried out using the procedure of Speckamp *et al*.[13] 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-O^{2}H 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 α-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 α-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 α-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.

Scheme 3

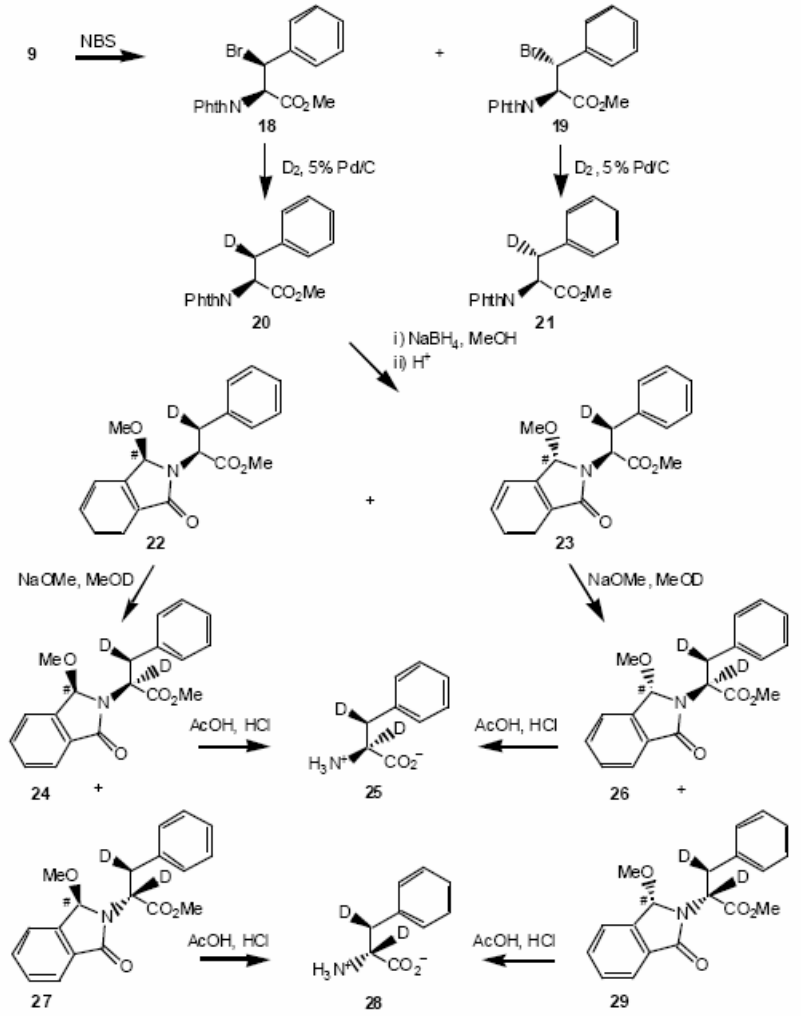
It was not necessary to assign the stereochemistry of the methoxy amides **10** and **11** at the 3-position of the isoindoline moiety in order to exploit the new chiral centre of these compounds, to distinguish and separate the stereoisomers of the deuterated derivatives **12**, **14**, **15** and **17** 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.[14] 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,[6] to give **20** and **21**, respectively. When the (2*S*,3*S*)-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 ^{1}H 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.

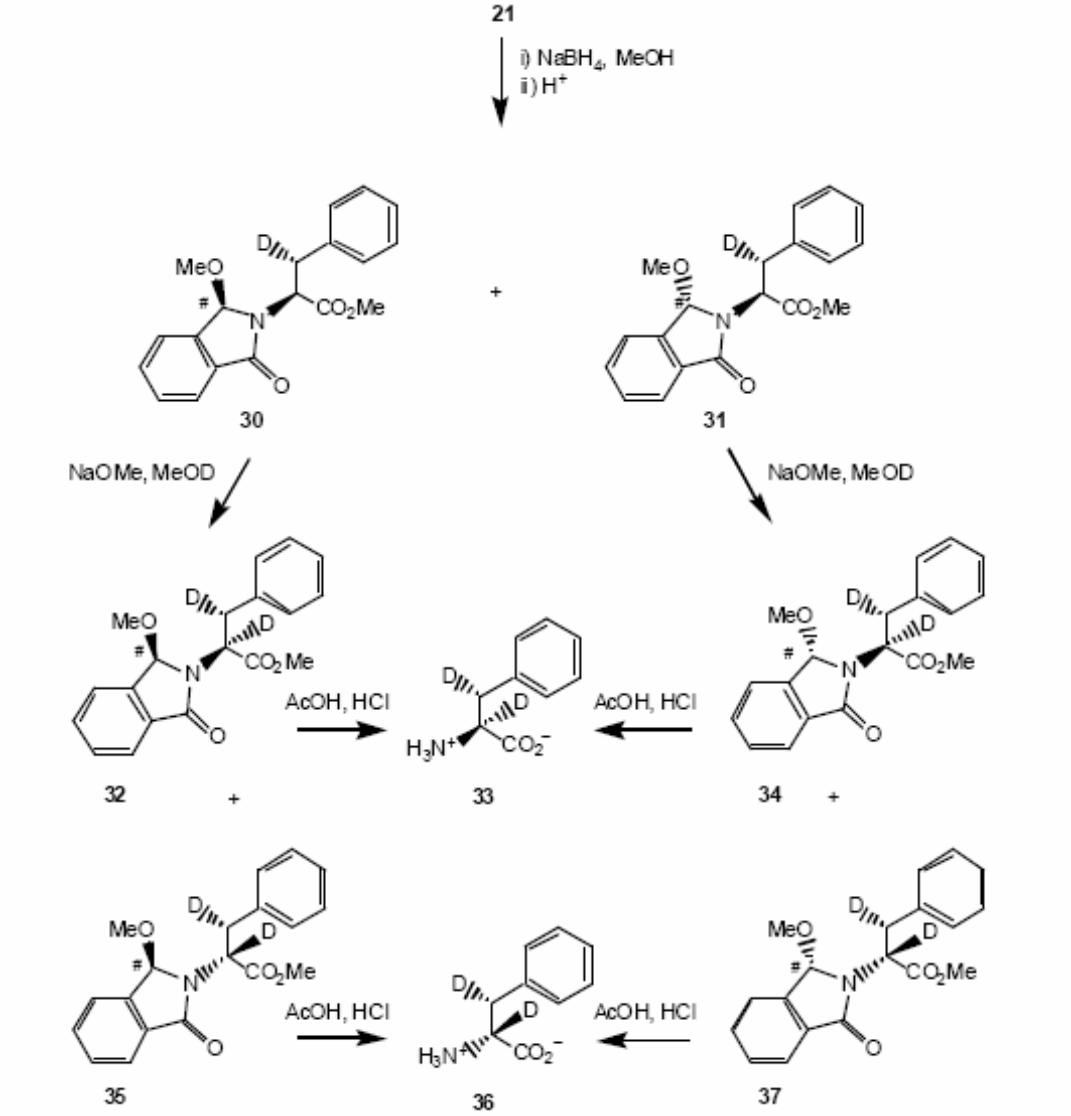
With the deuterated phenylalanines **13**, **16**, **25**, **28**, **33** and **36** in hand, their reactions with PAL in 0.04 mol L^{-1} sodium borate buffer (pH 8.7) at 30 C were investigated. The samples of *trans*-cinnamic acid **2** isolated from these reactions contained various amounts of deuterium, 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 α- or β-position. Reaction at the β-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).



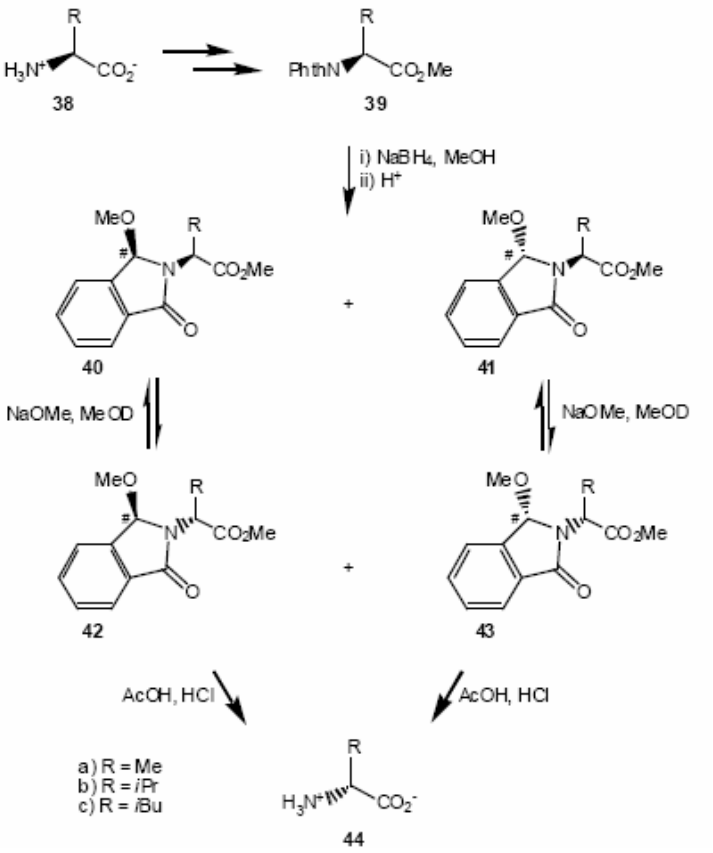
[#] The stereochemistry at this position has been assigned arbitrarily and may be the reverse, although compounds **22**, **24** and **27** must have the same stereochemistry at the C3'-position, as must compounds **23**, **26** and **29**.

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

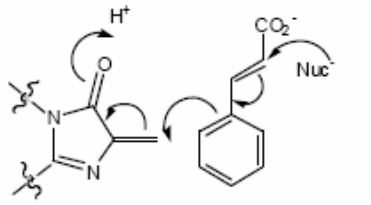


Figure 1. Activation of the cinnamate **2** by PAL to nucleophilic addition at the α-position.

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.[12] 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.[6,14] 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 • 10^3 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 ^{1}H 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 (2*S*,3'*R*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenyl-propanoate (10) and methyl (2*S*,3'*S*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-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 *R*t 12.5 min; ^{1}H 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 (42), 132 (100); and 11 (460 mg, 31%) (Found: C, 70.20; H, 5.69; N, 4.27. $C_{19}H_{19}NO_4$ requires C, 70.14; H, 5.89; N, 4.30%); HPLC R_t 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 (2*S*,3*S*,3'*R*)-[3-^{2}H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenyl-propanoate (22) and methyl (2*S*,3*S*,3'*S*)-[3-$^2H_1$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (23). Reaction of (2*S*,3*S*)-[3-$^2H_1$]-*N*-phthaloylphenylalanine methyl ester **20** afforded the title compounds **22**; HPLC R_t 12.5 min; ^{1}H 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% ^{2}H1); and **23**; HPLC *R*t 13.5 min; ^{1}H 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% $^2H_1$).

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 (2*S*,3*R*,3'*R*)-[3-^{2}H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (30) and methyl (2*S*,3*R*,3'*S*)-[3-$^2H_1$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (31). Reaction of (2*S*,3*R*)-[3-$^2H_1$]-*N*-phthaloylphenylalanine methyl ester **21** afforded the title compounds **30**; HPLC R_t 12.5 min; ^{1}H NMR δ 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% $^2H_1$); and **31**; HPLC R_t 13.5 min; ^{1}H NMR δ 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% $^2H_1$). 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 (2*S*,3'*R*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-propanoate (40a) and methyl (2*S*,3'*S*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-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 δ 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 δ 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 (2*S*,3'*R*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-methyl-butanoate (40b) and methyl (2*S*,3'*S*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-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 δ 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 δ 1.04 (3H, d, *J* = 7 Hz), 1.07 (3H, d, *J* = 7 Hz), 2.60 (1H, m), 3.09 (3H, s), 3.72 (3H, s), 4.39 (1H, d, *J* = 9 Hz), 6.11 (1H, s), 7.5–7.9 (4H, m); MS *m/z*: 277 (M^+, 27%), 245 (18), 218 (46), 132 (100).

Methyl (2*S*,3'*R*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-4-methyl-pentanoate (40c) and methyl (2*S*,3'*S*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-4-methylpentanoate (41c). Reaction of (*S*)-*N*-phthaloylleucine methyl ester **39c** afforded the title compounds **40c** (396 mg, 30%); HPLC R_t 10.5 min; ^{1}H NMR δ 0.96 (3H, d, *J* = 7 Hz), 1.22 (3H, d, *J* = 7 Hz), 1.65 (1H, m), 1.92 (1H, m), 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 δ 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 a-methoxy amides 10, 11, 22, 23, 30 and 31. A solution prepared from sodium (48 mg, 2 mmol) and methanol-O^{2}H (1 mL) was added to a solution of the α-methoxy amide (1 mmol) in methanol-O^{2}H (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 (2*S*,3'*R*)-[2-^{2}H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (12) and methyl (2*R*,3'*R*)-[2-^{2}H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (15). Reaction of methyl (2*S*,3'*R*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate **10** afforded the title compounds **12**; HPLC R_t 12.5 min; ^{1}H NMR δ 2.84 (3H, s), 3.50 (2H, broad s), 3.77 (3H, s), 5.10 (1H, s), 7.1–7.9 (9H, m); MS *m/z*: 326 (M$^+$, 96% ^{2}H$_1$); and 15; HPLC R_t 13.5 min; ^{1}H NMR δ 2.40 (3H, s), 3.61 (1H, d, *J* = 14.5 Hz), 3.72 (d, *J* = 14.5 Hz), 3.75 (3H, s), 5.81 (1H, s), 7.1–7.9 (9H, m); MS *m/z*: 326 (M$^+$, 95% ^{2}H$_1$). Their other properties were comparable with those of the unlabelled analogue **10** and the unlabelled enantiomer **11**.

Methyl (2*S*,3'*S*)-[2-^{2}H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (14) and methyl (2*R*,3'*S*)-[2-^{2}H$_1$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (17). Reaction of methyl (2*S*,3'*S*)-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate **11** afforded the title compounds **14**; HPLC R_t 13.5 min; ^{1}H NMR δ 2.41 (3H, s), 3.60 (1H, d, *J* = 14 Hz), 3.72 (d, *J* = 14 Hz), 3.75 (3H, s), 5.79 (1H, s), 7.1–7.9 (9H, m); MS *m/z*: 326 (M$^+$, 96% ^{2}H1); and **17**; HPLC R_t 12.5 min; ^{1}H NMR δ 2.85 (3H, s), 3.50 (2H, broad s), 3.77 (3H, s), 5.11 (1H, s), 7.1–7.9 (9H, m); MS *m/z*: 326 (M$^+$, 94% ^{2}H$_1$). Their other properties were comparable with those of the unlabelled analogue **11** and the unlabelled enantiomer **10**.

Methyl (2*S*,3*S*,3'*R*)-[2,3-^{2}H2]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (24) and methyl (2*R*,3*S*,3'*R*)-[2,3-^{2}H$_2$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (27). Reaction of methyl (2*S*,*3S*,*3*'*R*)-[3-^{2}H1]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate **22** afforded the title compounds **24**; HPLC R_t 12.5 min; ^{1}H NMR δ 2.83 (3H, s), 3.50 (1H, s), 3.78 (3H, s), 5.09 (1H, s), 7.1–7.9 (9H, m); MS *m/z*: 327 and 326 (M$^+$, 93% ^{2}H2, 99% ^{2}H$_1$); and **27**; HPLC *R*t 13.5 min; ^{1}H NMR δ 2.40 (3H, s), 3.70 (1H, s), 3.75 (3H, s), 5.82 (1H, s), 7.1–7.9 (9H, m); MS *m/z*: 327 and 326 (M$^+$, 92% ^{2}H$_2$, 98% ^{2}H$_1$). The deuterides **24** and **27** were stereochemically pure, within the limits of detection using ^{1}H NMR spectroscopy (>95%). Their other properties were comparable with those of the unlabelled analogue **10** and the unlabelled enantiomer **11**.

Methyl (2*S*,3*S*,3'*S*)-[2,3-^{2}H2]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (26) and methyl (2*R*,3*S*,3'*S*)-[2,3-^{2}H$_2$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-

isoindol-2-yl)-3-phenylpropanoate (29). Reaction of methyl (2*S* ,*3S*,*3'S*)-[3-$^2H_1$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate **23** afforded the title compounds **26**; HPLC R_t 13.5 min; ^{1}H 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% ^{2}H2, 98% $^2H_1$); and 29; HPLC R_t 12.5 min; ^{1}H 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% $^2H_2$, 99% $^2H_1$). The deuterides **26** and **29** were stereochemically pure, within the limits of detection using ^{1}H NMR spectroscopy (>95%). Their other properties were comparable with those of the unlabelled analogue **11** and the unlabelled enantiomer **10**.

Methyl (2*S*,3*R*,3'*R*)-[2,3-^{2}H2]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (32) and methyl (2*R*,3*R*,3'*R*)-[2,3-$^2H_2$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (35). Reaction of methyl (2*S*,*3R*,*3'R*)-[3-$^2H_1$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate **30** afforded the title compounds **32**; HPLC R_t 12.5 min; ^{1}H 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% ^{2}H2, 98% $^2H_1$); and **35**; HPLC R_t 13.5 min; ^{1}H 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% $^2H_2$, 99% $^2H_1$). The deuterides **32** and **35** were stereochemically pure, within the limits of detection using ^{1}H NMR spectroscopy (>95%). Their other properties were comparable with those of the unlabelled analogue **10** and the unlabelled enantiomer **11**.

Methyl (2*S*,3*R*,3'*S*)-[2,3-$^2H_2$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (34) and methyl (2*R*,3*R*,3'*S*)-[2,3-$^2H_2$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate (37). Reaction of methyl (2*S*,*3R*,3'*S*)-[3-$^2H_1$]-2-(2,3-dihydro-3-methoxy-1-oxo-1*H*-isoindol-2-yl)-3-phenylpropanoate **31** afforded the title compounds **34**; HPLC R_t 13.5 min; ^{1}H 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% $^2H_2$, 99% $^2H_1$); and **37**; HPLC R_t 12.5 min; ^{1}H 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% $^2H_2$, 98% $^2H_1$). The deuterides **34** and **37** were stereochemically pure, within the limits of detection using ^{1}H 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 a-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 αmethoxy 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 ^{1}H 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 diastereochemically pure, within the limits of detection using ^{1}H NMR spectroscopy (>95%).

(2*S*)-[2-^{2}H$_1$]-Phenylalanine (13). Prepared from **12** and **14**, the title compound **13** had ^{1}H NMR δ (^2H_2O) 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% $^2H_1$).

(2*R*)-[2-^{2}H$_1$]-Phenylalanine (16). Prepared from **15** and **17**, the title compound **16** had ^{1}H NMR δ (^2H_2O) 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% $^2H_1$).

(2*S*,3*S*)-[2,3-^{2}H$_2$]-Phenylalanine (25). Prepared from **24** and **26**, the title compound **25** had ^{1}H NMR δ (^2H_2O) 3.02 (1H, s), 7.2–7.4 (5H, m); MS *m/z*: 168 and 167 (M^+ + 1, 94% ^{2}H2, 98% $^2H_1$).

(2*R*,3*S*)-[2,3-^{2}H$_2$]-Phenylalanine (28). Prepared from **27** and **29**, the title compound **28** had ^{1}H NMR δ (^2H_2O) 3.20 (1H, s), 7.2–7.4 (5H, m); MS *m/z*: 168 and 167 (M^+ + 1, 93% $^2H_2$, 99% $^2H_1$).

(2*S*,3*R*)-[2,3-^{2}H$_2$]-Phenylalanine (33). Prepared from **32** and **34**, the title compound **33** had ^{1}H NMR δ (^2H_2O) 3.21 (1H, s), 7.2–7.4 (5H, m); MS *m/z*: 168 and 167 (M^+ + 1, 95% $^2H_2$, 98% $^2H_1$).

(2*R*,3*R*)-[2,3-^{2}H2]-Phenylalanine (36). Prepared from **35** and **37**, the title compound **36** had ^{1}H NMR δ (^2H_2O) 3.03 (1H, s), 7.2–7.4 (5H, m); MS *m/z*: 168 and 167 (M^+ + 1, 93% $^2H_2$, 99% $^2H_1$).

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**; ^{1}H 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: ^{1}H 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% $^2H_2$, 95% $^2H_1$).

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