Synthesis of L-*threo*- and L-*erythro*-[1-^{13}C, 2,3-$^{2}H_2$]amino acids: novel probes for conformational analysis of peptide side chains

Makoto Oba,[a] Ryuichi Ueno,[b] Mika Fukuoka (nee Yoshida),[b] Masatsune Kainosho[b] and Kozaburo Nishiyama *,[a]

[a] *Department of Meterial Science and Technology, Tokai University, 317, Nishino, Numazu, Shizuoka 410-03, Japan*
[b] *Department of Chemistry, Tokyo Metropolitan University, 1-1, Minamiohsawa, Hachioji, Tokyo 192-03, Japan*

An efficient and convenient route for the preparation of L-*threo*- and L-*erythro*-[1-^{13}C, 2,3-$^{2}H_2$]amino acids **5** as probes for the conformational analysis of peptide side chains by NMR spectroscopy is described. Stereoselective incorporation of deuterium into the α,β-positions of amino acid **5** was accomplished by catalytic deuteriation of dehydroamino acid derivatives **1** and **2** followed by a combination of enzymic optical resolution and the racemization at the 2-position. Using these doubly labelled amino acids, it was possible to obtain vicinal coupling constants between carbonyl carbon and prochiral β-protons, $J(^{13}C^1-^1H^{\beta 1})$ and $J(^{13}C^1-^1H^{\beta 2})$, through ^{13}C NMR spectroscopy alone. We also demonstrate the determination of the fractional populations of rotamers in respect of the $C^\alpha-C^\beta$ bond of the amino acids using the measured coupling constants.

Introduction

Recently, NMR spectroscopic analyses of peptide molecules in solution have become more and more important, and indispensable for understanding their dynamic conformations. However, the NMR signals of high-molecular-mass peptides are generally too broad and complex to be assigned properly. To resolve these problems, 2D and 3D NMR methodologies have been developed extensively.[1] Also, positive (^{13}C, ^{15}N) and negative (^{2}H) stable-isotope labelling are considered essential techniques for this purpose. On the other hand, the properties and functions of peptide molecules depend particularly on the conformation of not only the main chains but also the side chains. Information on the side-chain conformation has been gleaned mainly from observations of the nuclear Overhauser effect (NOE) and the spin–spin coupling constants. Coupling constants between vicinal nuclei on the $C^\alpha-C^\beta$ bond afford information about its torsion angle, χ^1, in the peptide. However, the assignment of these signals is seriously limited as they are not easily accessible from the complicated spectrum. In conformational studies on such side chains by NMR spectroscopy, the stable-isotope labelling method is considered to be a reliable technique for the extraction of the required information.[2-5] Since it was difficult to incorporate these labelling atoms into peptide molecules directly, we then attempted to synthesize some amino acids regio- and stereoselectively labelled with stable isotopes[6] and to incorporate such labelled amino acids into peptide molecules. In this paper, we report a preparation of L-*threo*- and L-*erythro*-[1-^{13}C, 2,3-$^{2}H_2$]amino acids as one of our recent interests in the synthetic field of labelled compounds. Using these amino acids, individual vicinal heteronuclear coupling constants between the carbonyl carbon and prochiral β-protons, $J(^{13}C^1-^1H^{\beta 1})$ and $J(^{13}C^1-^1H^{\beta 2})$, should be easily obtained because the carbonyl carbon is enriched by the ^{13}C atom and the complicating additional couplings are completely suppressed by stereoselective deuterium substitution at the α and β position, *i.e.* α- and β2-protons of the *threo* isomer are substituted with deuterium (Fig. 1). Therefore, a determination of the fractional populations for three staggered rotamers **I**, **II** and **III** in respect of the $C^\alpha-C^\beta$ bond should be feasible using the measured coupling constants, according to Pachler's equations.[7] Note that it is possible to obtain information about angle χ^1 from the ^{13}C NMR spectrum alone, and, hence, these doubly labelled amino acids can be regarded as new probes for the determination of angle χ^1 even in a large peptide molecule. The present work also demonstrates the first example of the estimation of rotamer population about $C^\alpha-C^\beta$ bond in amino acids through ^{13}C NMR spectroscopy.

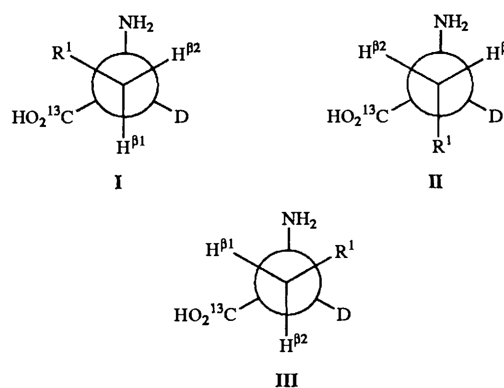
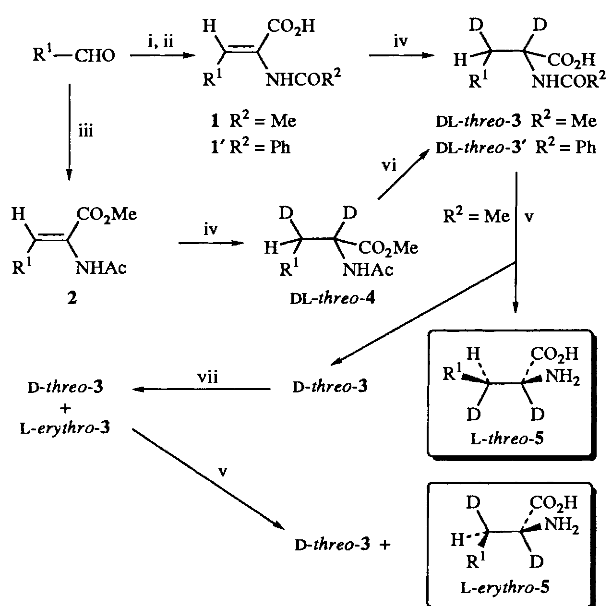


Fig. 1 Newman projection of three staggered rotamers of amino acid side chain

Results and discussion

In order to introduce deuterium atoms stereoselectively into the α- and β-position of an amino acid, we examined a catalytic deuteriation of the corresponding dehydroamino acid followed by enzymic optical resolution. A concise synthetic route to L-*threo*- and L-*erythro*-[2,3-$^{2}H_2$]amino acids **5** is illustrated in Scheme 1. First of all, we reinvestigated the preparation of (*Z*)-*N*-acyldehydroamino acids from *N*-acylglycine and appropriate aldehydes according to the Erlenmeyer method,[8] because a systematic examination of these reactions had not apparently appeared in the literature, in particular the reaction of *N*-acetylglycine with aliphatic and heterocyclic aldehydes. The yields of *N*-acetyl- (**1a–e**) and *N*-benzoyl-dehydroamino acids (**1a′–e′**) from *N*-acetylglycine or *N*-benzoylglycine with the corresponding aldehyde (2 steps) are listed in Table 1. In the presence of sodium acetate, condensation of benzaldehyde, 4-hydroxybenzaldehyde, and indole-3-carbaldehyde with *N*-



Scheme 1 *Reagents and conditions:* i, $R^2CONHCH_2CO_2H$, AcONa, Ac_2O (120 °C; 2 h for R^2 = Me; 100 °C; 1 h for R^2 = Ph); ii, 0.2 mol dm^{-3} aq. Na_2CO_3, reflux, 4 h; iii, $(MeO)_2POCH(NHAc)CO_2Me$, DBU; iv, D_2, catalyst; v, acylase, 37 °C, 48–72 h; vi, 2.5 mol dm^{-3} aq. NaOD–MeOD; vii, 2.5 mol dm^{-3} aq. NaOD, Ac_2O, 70 °C

Table 1 Yields of (*Z*)-*N*-acyldehydroamino acid **1** obtained by the Erlenmeyer method

Run	R^1	R^2	Yield of **1** (%)
1	Ph	Me	80 (**a**)
2	Ph	Ph	69 (**a′**)
3	4-Hydroxyphenyl	Me	76 (**b**)
4	4-Hydroxyphenyl	Ph	71 (**b′**)
5	Indol-3-yl	Me	77 (**c**)
6	Indol-3-yl	Ph	38 (**c′**)
7	Imidazol-4-yl	Me	0 (**d**)
8	Imidazol-4-yl	Ph	42 (**d′**)
9	Me_2CD	Me	0 (**e**)
10	Me_2CD	Ph	21 (**e′**)

acetyl- or *N*-benzoyl-glycine in acetic anhydride occurred to give the azlactones, which were decomposed by alkaline hydrolysis to afford the corresponding (*Z*)-*N*-acyldehydroamino acids **1a–c** and **1a′–c′** in moderate to good yields (entries 1–6), respectively. Similar treatment of imidazole-4-carbaldehyde with *N*-benzoylglycine gave (*Z*)-2-benzamido-3-(imidazol-4′-yl)propenoic acid **1d′**. However, the *N*-acetyldehydroamino acid **1d** was not obtained by the reaction of imidazole-4-carbaldehyde with *N*-acetylglycine. It is known that the preparation of *N*-acyldehydroamino acids for practical purposes is limited to only aromatic aldehydes in the Erlenmeyer reaction. In fact, although the condensation of 2-methyl-[2-^{2}H]propanal, prepared from 2-methylpropanal with D_2O at 170 °C in a sealed tube,[9] with *N*-benzoylglycine gave 2-benzamido-4-methyl[4-^{2}H]pent-2-enoic acid **1e′** in 21% yield, similar condensation with *N*-acetylglycine resulted in no production of the corresponding acetamide **1e**. Since *N*-acetyl derivatives were important for subsequent enzymic optical resolution (*vide infra*), we also adopted the phosphorylglycine method[10] for the preparation of *N*-acetyldehydroamino acid derivatives which could not be obtained by the Erlenmeyer method. Methyl *N*-acetyl-2-(dimethoxyphosphoryl)glycinate[10] was prepared from *N*-acetylglycine by a modified Kober and Steglich procedure,[11] and was then condensed with 1-tritylimidazole-4-carbaldehyde and 2-methyl-[2-^{2}H]propanal in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford the corresponding methyl *N*-acetyldehydroamino acid esters **2d** and **2e** in 49 and 61% overall yield (4 steps from *N*-acetylglycine), respectively. In contrast to Schmidt's report,[12] only the (*Z*)-isomer was detected by an analysis of the reaction mixture using ^{1}H NMR spectroscopy.

Generally, deuteriation of the (*Z*)-isomer should give only the *threo* isomer since catalytic hydrogenation of olefins proceeds *via* a *cis* addition. The catalytic deuteriation of (*Z*)-*N*-acetyldehydroamino acids **1** and **2** was carried out using a transition metal catalyst and deuterium gas in a deuteriated solvent under medium pressure (5 kgf/cm^2). A thorough analysis of the deuteriated product by means of ^{1}H NMR (400 MHz) spectroscopy showed the formation of the *threo* isomer as a major product together with a small amount of the *erythro* isomer. The ratio of the stereoisomers formed depended largely upon the catalyst and the reactant. The conditions, yields, and stereoselectivities of the reactions are compiled in Table 2. Typically, a mixture of (*Z*)-2-acetamido-3-phenylpropenoic acid **1a** (4.88 mmol) and 10% Pd/C (20 w/w%) in MeOD (20 cm^3) was deuteriated for 24 h to give DL-*threo*-*N*-acetyl-[2,3-$^2H_2$]phenylalanine **3a**, along with a small amount of DL-*erythro*-isomer, in quantitative yield. The molar ratio of *threo* to *erythro* isomers was determined to be 99:1 by ^{1}H NMR analysis. While the deuteriation of (*Z*)-2-acetamido-3-(4′-hydroxyphenyl)propenoic acid **1b** by using 10% Pd/C also proceeded diastereoselectively to give a mixture of DL-*threo*- and DL-*erythro*-*N*-acetyl-[2,3-$^2H_2$]tyrosine **3b** (*threo*:*erythro* >99:1), the diastereoisomer ratio obtained for *N*-acetyl-[2,3-$^2H_2$]tryptophan **3c**, which was the deuteriation product of 2-acetamido-3-(indol-3′-yl)propenoic acid **1c**, was slightly lower. The use of the Wilkinson catalyst, $RhCl(PPh_3)_3$, improved the ratio of *threo*:*erythro* isomers for product **3c** to 98:2. In the case of 2-benzamido-3-(imidazol-4′-yl)propenoic acid **1d′**, the diastereoselectivity of the deuterium addition was significantly lower, at 85:15 (*threo*:*erythro*), probably due to an interconversion between *E* and *Z* isomers of the dehydroamino acid during the reduction. Such low-stereoselectivity compounds are not suitable for conformational studies of amino acids and peptide molecules by NMR spectroscopy. For compound **1d′**, $RhCl(PPh_3)_3$ did not promote the reaction under the conditions used. On the other hand, the deuteriation of methyl 2-acetamido-3-(1′-tritylimidazol-4′-yl)propenoate **2d** in the presence of 10% Pd/C furnished a diastereoisomeric mixture of methyl *N*-acetyl-[2,3-$^2H_2$]histidinate **4d** in quantitative yield, and the ratio of *threo*:*erythro* isomers was found to be 98:2. On the deuteriation of 2-benzamido-4-methyl-[4-^{2}H]pent-2-enoic acid **1e′** in MeOD with 10% Pd/C as catalyst, considerable H/D scrambling was exhibited in the obtained product *N*-benzoyl-[2,3,4-$^2H_3$]leucine **3e′** by ^{1}H NMR analysis of the product. The Wilkinson-type catalysts, $RhCl(PPh_3)_3$ and $RuCl_2(PPh_3)_3$, were effective for diastereoselective deuteriation without deuterium scrambling. Furthermore, $RhCl(PPh_3)_3$ catalysed the quantitative deuteriation of methyl 2-acetamido-4-methyl[4-^{2}H]pent-2-enoate **2e** to afford methyl *N*-acetyl-[2,3,4-$^2H_3$]leucinate **4e** (*threo*:*erythro* >99:1). For compound **2e**, the previous deuterium labelling at the 4-position was necessary in order to cancel the spin–spin coupling and overlapping between the β- and γ-proton signals in product **4e**.

For an optical resolution of (3*R*)- and (3*S*)-isomers, asymmetric enzymic hydrolysis of racemic *N*-acyl-[2,3-$^2H_2$]amino acids using acylase would be expected to be possible. It is an important key step described in this paper. For example, the action of *Aspergillus* acylase on DL-*threo*-*N*-acetylphenylalanine **3a**, a mixture of (2*S*,3*S*)- and (2*R*,3*R*)-form, in the presence of $CoCl_2$ at 37 °C for 48–72 h yielded L-

Table 2 Catalytic deuteriation of dehydroamino acids **1** and **2**

Run	Dehydroamino acid	Catalyst[a]	Solvent	Product Yield (%)	*threo*:*erythro*[b]	
1	**1a**	Pd	MeOD	91	99:1	**3a**
2	**1a′**	Pd	MeOD	100	99:1	**3a′**
3	**1b**	Pd	MeOD	100	>99:1	**3b**
4	**1b′**	Pd	MeOD	100	96:4	**3b′**
5	**1c**	Pd	MeOD	100	94:6	**3c**
6	**1c**	Rh	MeOD + C_6H_6	82	98:2	**3c**
7	**1c′**	Pd	MeOD	100	91:9	**3c′**
8	**1c′**	Rh	MeOD + C_6H_6	73	96:4	**3c′**
9	**1d′**	Pd	AcOD	100	85:15	**3d′**
10	**1d′**	Rh	AcOD + C_6H_6	0		**3d′**
11	**2d**	Pd	AcOD	100	98:2	**4d**
12	**1e′**	Pd	MeOD	100	H–D scrambling	**3e′**
13	**1e′**	Rh	MeOD + C_6H_6	85	98:2	**3e′**
14	**1e′**	Ru	MeOD + C_6H_6	88	97:3	**3e′**
15	**2e**	Rh	MeOD + C_6H_6	98	>99:1	**4e**

[a] Pd: 10% Pd/C (20 wt%), Rh: $RhCl(PPh_3)_3$ (2 mol%), Ru: $RuCl_2(PPh_3)_3$ (2 mol%). [b] Determined by ^1H NMR spectroscopy.

Table 3 Yields and spectral data of L-*threo*- and L-*erythro*-[2,3-$^2H_2$]amino acid **5**

Amino acid	Configuration of β-position	Yield (%)[a]	de (%)[b]	ee (%)[c]	Chemical shift of β-proton (δ_H)
L-*threo*-**5a**	*S*	85	97	100	3.09
L-*erythro*-**5a**	*R*	69	95	100	3.25
L-*threo*-**5b**	*S*	90	99	100 (99)	2.64
L-*erythro*-**5b**	*R*	61	89	100	2.82
L-*threo*-**5c**	*S*	90	95	100	3.27
L-*erythro*-**5c**	*R*	68	86	100	3.45
L-*threo*-**5d**	*R*	99	94	100	3.08
L-*erythro*-**5d**	*S*	23	89	100	3.20
L-*threo*-**5e**	*S*	84	98	100 (100)	1.64
L-*erythro*-**5e**	*R*	77	87	100 (99)	1.70

[a] Recovery of L-form. [b] Ratio of *threo*:*erythro* was determined by ^1H NMR spectroscopy. [c] Determined by HPLC analysis. The values obtained by optical-rotation values are also shown in parentheses. The ee-value refers to C(2).

threo-[2,3-$^2H_2$]phenylalanine **5a** in addition to unchanged D-*threo*-**3a**. Optical resolution at the 3-position as well as at the 2-position was accomplished by exclusively L-directed hydrolysis of the N-acetyl group. In order to obtain L-*erythro*-[2,3-$^2H_2$]phenylalanine **5a** from the remaining D-*threo*-**3a**, the latter was racemized at the 2-position to give a mixture of L-*erythro*- and D-*threo*-**3a** by treatment with an excess of acetic anhydride, and the product was subjected to stereospecific hydrolysis to give L-*erythro*-**5a** as required. We found that the acylase was not able to hydrolyse *N*-benzoyl derivatives **3′** efficiently under the conditions employed. In the case of esters **4d** and **4e**, these were converted into acids **3d** and **3e** by hydrolysis of the methyl ester with 2.5 mol dm^{-3} NaOD in MeOD before the optical-resolution stage. For acid **3d**, *Aspergillus* acylase did not work well but porcine kidney acylase was found to be effective, and repeated treatments with the acylase should give complete optical resolution. The results are summarized in Table 3. The absolute configuration at the 2,3-positions of the L-*threo*- and the L-*erythro*-isomers are (2*S*,3*S*) and (2*S*,3*R*) for amino acids **5a, 5b, 5c** and **5e**, but (2*S*,3*R*) and (2*S*,3*S*) for compound **5d**. While the isolated yields of the *threo*-isomers were excellent, those of the *erythro*-isomers were moderate since the yields are overall values for three steps (resolution–racemization–resolution) from substrates DL-*threo*-**3**. The diastereoisomeric excess (de) was calculated from the relative NMR areas of the β-proton signals of the *threo*- and the *erythro*-isomers. As compared with the high de-values for the *threo*-isomers, the slightly lower diastereoselectivity observed in the *erythro*-isomers was rationalized by assuming an incomplete hydrolysis of L-*threo*-*N*-acetyl derivatives by the acylase in the first resolution step. The enantiomeric excess of the amino acids **5** was determined to be 100% by high-performance liquid chromatography (HPLC) with a chiral stationary-phase column. The ^1H NMR spectra of amino acids stereoselectively labelled with deuterium are remarkably simple and the chemical sifts of the prochiral β-protons are also compiled in Table 3. The signal of β_1-proton (detected in the *threo*-form) usually resonated at higher field compared with that of the β_2-proton which is observed in the *erythro*-form. The configurational notations of β_1- and β_2-protons are *pro-R* and *pro-S* for phenylalanine, tyrosine, tryptophan and leucine but *pro-S* and *pro-R* for histidine. As the β-proton signals of these amino acids are singlets these compounds are considered to be useful for stereospecific assignment of prochiral β-protons and NOE studies in peptides.

These results prompted us to examine the preparation of doubly labelled amino acids in which the carbonyl carbon was enriched with ^{13}C. The starting [1-^{13}C]glycine is commercially available or can be easily obtained by bromination of [1-^{13}C]acetic acid followed by amination. After acetylation of amino group, *N*-acetyl[1-^{13}C]dehydroamino acid **1** and the corresponding methyl ester [1-^{13}C]-**2** were prepared by the modified Erlenmeyer method and phosphorylglycine method, respectively. Then, [1-^{13}C]-**1** or [1-^{13}C]-**2** was deuteriated to DL-*threo*-*N*-acetyl[1-^{13}C, 2,3-$^2H_2$]amino acid **3** or its methyl ester **4** followed by optical resolution to afford L-*threo*-[1-^{13}C, 2,3-$^2H_2$]amino acid **5** and L-*erythro*-[1-^{13}C]-**5**, similar to

Table 4 Rotamer populations of amino acids in D_2O

Amino acids	Coupling constants (Hz)		Population (P)[a]		
	$J(C^1–H^{\beta 2})$	$J(C^1–H^{\beta 1})$	I	II	III
Phe	3.8	2.6	0.30 (0.28)	0.15 (0.24)	0.55 (0.48)[b]
Tyr	4.0	2.4	0.32 (0.33)	0.13 (0.22)	0.55 (0.46)[b]
Trp	3.9	1.5	0.31	0.02	0.67
His	3.8	2.2	0.31	0.11	0.58
Leu	3.0	3.1	0.20	0.21	0.59

[a] Calculated by Pachler's equations (ref. 7): $P_I = [J(C^1–H^{\beta 2}) - J_g]/(J_t - J_g)$, $P_{II} = [J(C^1–H^{\beta 1}) - J_g]/(J_t - J_g)$, $P_{III} = 1 - (P_I + P_{II})$, in which J_t = 9.8 Hz and J_g = 1.3 Hz (ref. 3). [b] Ref. 2.

compounds **1** or **2** in Scheme 1. In order to obtain the structural proof of these doubly labelled amino acids, we measured the individual vicinal $^{13}C–^1H$ coupling constants between the carbonyl carbon and the prochiral β-protons from ^{13}C NMR spectra, and we also calculated the fractional rotamer populations about the $C^\alpha–C^\beta$ bond from the measured heteronuclear vicinal coupling constants using Pachler's equations.[7] The values of $J(^{13}C^1–^1H^{\beta 1})$ and $J(^{13}C^1–^1H^{\beta 2})$, and the calculated rotamer populations are given in Table 4. These results show a predominance of conformation **III** in which the side chain and the carboxylic function are in a *trans* conformation. A good agreement between these results and those obtained from $^1H^\alpha–^1H^\beta$ coupling constants, which one of us previously reported,[2] shows the applicability of the $^{13}C^1–^1H^\beta$ coupling constants to the determination of fractional rotamer populations. The ^{13}C-detected proton-coupled NMR spectra offer several advantages for the conformational studies of the side chain, especially in large peptide molecules. Only the required coupling constants, $J(^{13}C^1–^1H^{\beta 1})$ and $J(^{13}C^1–^1H^{\beta 2})$, can be measured according to the assortment of deuterium substitution even if the two prochiral β-protons cannot be assigned or they have extremely close chemical shifts.

In conclusion, the present route provides an efficient method for the chemical synthesis of L-*threo*- and L-*erythro*-[1-^{13}C, 2,3-$^2H_2$]amino acids. These doubly labelled amino acids are shown to exclude completely the complexity in the β-proton region of the ^1H NMR spectrum to facilitate the unequivocal assignment of the prochiral β-protons. Furthermore, the information about the $C^\alpha–C^\beta$ torsion angle can be obtained from the carbonyl carbon signals alone and the compounds are, therefore, suitable for use as analytical probes for a conformational study of peptide side chains.

Experimental

Mps were determined on a Yamato MP-21 melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, $(CD_3)_2SO$ or D_2O on a Varian UNITY-400 spectrometer. All chemical shifts are reported as δ-values (ppm) relative to residual chloroform (δ_H 7.26), tetramethylsilane (δ_H 0), sodium 3-(trimethylsilyl)propanesulfonate (δ_H 0), or 1,4-dioxane (δ_C 66.5). *J*-Values are given in Hz. High-resolution mass spectra (EI) were obtained on a JEOL JMS-AX-500 spectrometer with DA7000 data system using perfluorokerosene as an internal standard. For ion-exchange chromatography, DOWEX 50W-X8 or Amberlite IRC-50 activated with 1 mol dm^{-3} HCl was used. Optical purities were determined on a Senshu SSC-3100 high-pressure liquid chromatography system equipped with a chiral MCIGEL CRS10W column from Mitsubishi Kasei Co. and 0.1 or 2 mmol dm^{-3} aq. $CuSO_4$ solution as eluent. Catalytic hydrogenation was performed in an Ishii CHA-S medium-pressure catalytic hydrogenator.

2-Methyl[2-^2H]propanal[9] and 1-tritylimidazole-4-carbaldehyde[13] were synthesized according to the reported procedure. $RhCl(PPh_3)_3$[14] and $RuCl_2(PPh_3)_3$[15] were prepared according to a general procedure. All other reagents were of commercial grade and used as supplied.

Preparation of dehydroamino acids

A mixture of indole-3-carbaldehyde (4.35 g, 30 mmol), *N*-acetylglycine (2.34 g, 20 mmol), sodium acetate (2.46 g, 30 mmol), and acetic anhydride (12.24 g, 120 mmol) was heated at 120 °C for 2–3 h. After cooling of the reaction mixture, the resulting solid mass was treated with water (5 cm^3) and then broken up. The crystals were then collected by suction and washed successively with water and then a small amount of EtOH to afford the crude (*Z*)-azlactone of acid **1c**.

The crude azlactone was hydrolysed in refluxing 0.2 mol dm^{-3} aq. Na_2CO_3 (120 cm^3) for 4 h and the resulting clear solution was acidified with conc. HCl. The precipitated solid was collected, washed with water, and dried over $CaCl_2$ in a vacuum desiccator to give almost pure (*Z*)-2-*acetamido*-3-(*indol*-3′-*yl*)*propenoic acid* **1c** (3.771 g, 77%); $\delta_H[(CD_3)_2SO]$ 2.04 (3 H, s), 7.16 (2 H, m), 7.46 (1 H, d, *J* 7.9), 7.64 (1 H, s), 7.72 (1 H, d, *J* 7.8), 7.81 (1 H, d, *J* 2.7), 9.21 (1 H, s), 11.72 (1 H, s) and 12.27 (1 H, br s) (Found: M^+, 244.0848. $C_{13}H_{12}N_2O_3$ requires *M*, 244.0848).

Other dehydroamino acids, (*Z*)-2-acetamido-3-phenylpropenoic acid **1a**,[16] (*Z*)-2-benzamido-3-phenylpropenoic acid **1a′**,[17] (*Z*)-2-acetamido-3-(4′-hydroxyphenyl)propenoic acid **1b**,[18] (*Z*)-2-benzamido-3-(4′-hydroxyphenyl)propenoic acid **1b′**,[19] (*Z*)-2-benzamido-3-(indol-3′-yl)propenoic acid **1c′**,[20] and (*Z*)-2-benzamido-3-(imidazol-4′-yl)propenoic acid **1d′**[21] were prepared similarly. In the case of *N*-benzoyl derivatives **1a′–d′**, the condensation reaction was carried out at 100 °C for 1 h. The 1-^{13}C derivatives of acids **1a–c** were prepared from *N*-acetyl-[1-^{13}C]glycine in an analogous manner.

(*Z*)-2-Benzamido-4-methyl[4-^2H]pent-2-enoic acid 1e′. A mixture of 2-methyl[2-^2H]propanal (6.56 g, 90 mmol), hippuric acid (5.37 g, 30 mmol), sodium acetate (3.69 g, 45 mmol), and acetic anhydride (18.36 g, 180 mmol) was heated at 90 °C for 1 h. After removal of acetic anhydride under reduced pressure, the residue was extracted with CH_2Cl_2, washed with water, dried over $MgSO_4$, and chromatographed on silica gel. Elution with a mixture of hexane and AcOEt (99:1) gave the (*Z*)-azlactone of **1e′** (1.57 g, 24%) along with a small amount of (*E*)-isomer.

The (*Z*)-isomer was hydrolysed in refluxing 0.2 mol dm^{-3} aq. Na_2CO_3 (90 cm^3) for 4 h and the resulting clear solution was acidified with conc. HCl. The precipitated solid was collected by suction and washed with water to afford pure *compound* **1e′** (3.03 g, 87%); $\delta_H(CDCl_3)$ 1.10 (6 H, s), 6.75 (1 H, s), 7.39 (1 H, br s), 7.47 (2 H, m), 7.55 (1 H, m) and 7.86 (2 H, m) (Found: M^+, 234.1094. $C_{13}H_{14}{}^2HNO_3$ requires *M*, 234.1115).

Methyl *N*-acetyl-2-(dimethoxyphosphoryl)glycinate

The preparation of the title compound was performed

according to the modified procedure of Steglich.[11] To a suspension of *N*-acetylglycine (5.85 g, 50 mmol) in MeOH (15 cm^3) was added $SOCl_2$ (11.9 g, 100 mmol) dropwise at −10 °C over a period of 1 h. The resulting clear solution was stirred at room temp. for 1 h and the reaction mixture was evaporated to give methyl 2-acetamidoacetate in quantitative yield.

To a refluxing mixture of the methyl ester with azoisobutyronitrile (AIBN) (0.164 g, 1 mmol) in CCl_4 (100 cm^3) was added dropwise a solution of Br_2 (12 g, 75 mmol) in CCl_4 (70 cm^3) under irradiation of a tungsten lamp over a period of 1–2 h and the heating was continued for an additional 3 h. The supernatant was collected by decantation and the gummy residue was extracted with hot AcOEt. The combined extract and supernatant were concentrated under reduced pressure to afford crude methyl 2-acetamido-2-bromoacetate quantitatively.

The bromide was quickly treated with a solution of $P(OMe)_3$ (6.2 g, 50 mmol) in CH_2Cl_2 (50 cm^3) for 2 h and the solvent was then removed by evaporation to give crude methyl *N*-acetyl-2-(dimethoxyphosphoryl)glycinate (11.68 g, 98%). The phosphorylglycine was used for the following condensation without further purification. An analytical sample was obtained by recrystallization from AcOEt, mp 89–90 °C (lit.,[10] 88–89 °C); $\delta_H(CDCl_3)$ 2.08 (3 H, d, *J* 0.8), 3.80 (3 H, d, *J* 8.5), 3.82 (3 H, d, *J* 0.6), 3.83 (3 H, d, *J* 8.5), 5.23 (1 H, dd, *J* 8.9 and 22.2) and 6.56 (1 H, br d, *J* 8.2). The 1-^{13}C derivative was prepared similarly from *N*-acetyl[1-^{13}C]glycine; $\delta_H(CDCl_3)$ 2.08 (3 H, d, *J* 0.8), 3.80 (3 H, d, *J* 8.5), 3.82 (3 H, dd, *J* 0.6 and 3.9), 3.83 (3 H, d, *J* 8.5), 5.23 (1 H, ddd, *J* 8.0, 8.9 and 22.2) and 6.46 (1 H, br d, *J* 8.2).

Methyl (*Z*)-2-acetamido-3-(imidazol-4′-yl)propenoate 2d

The condensation of phosphorylglycine with aldehyde was carried out by using the modified procedure of Schmidt *et al.*[12] To a solution of above crude methyl *N*-acetyl-2-(dimethoxyphosphoryl)glycinate (10 mmol) in CH_2Cl_2 (20 cm^3) was added DBU (1.52 g, 10 mmol) and the solution was stirred at room temp. for 25 min. Then, 1-tritylimidazole-4-carbaldehyde (3.38 g, 10 mmol) was added and the reaction mixture was stirred overnight. After evaporation of the solvent, the residue was submitted to column chromatography on silica gel. Elution with hexane–AcOEt (1:1) gave *title compound* **2d** (2.198 g, 49% from *N*-acetylglycine), mp 227–228 °C (from hexane–AcOEt); $\delta_H(CDCl_3)$ 2.13 (3 H, s), 3.81 (3 H, s), 6.42 (1 H, br s), 6.90 (1 H, s), 7.12 (6 H, m), 7.36 (9 H, m), 7.48 (1 H, s) and 10.29 (1 H, br s) (Found: M^+, 451.1903. $C_{28}H_{25}N_3O_3$ requires *M*, 451.1896).

Similarly, crude methyl *N*-acetyl-2-(dimethoxyphosphoryl)[1-^{13}C]glycinate (20 mmol) was treated with 1-tritylimidazole-4-carbaldehyde (6.76 g, 20 mmol) to afford *compound* [1-^{13}C]-**2d** (4.46 g, 49% from *N*-acetyl[1-^{13}C]glycine); $\delta_H(CDCl_3)$ 2.13 (3 H, s), 3.81 (3 H, d, *J* 3.9), 6.42 (1 H, br d, *J* 4.4), 6.90 (1 H, s), 7.12 (6 H, m), 7.36 (9 H, m), 7.48 (1 H, s) and 10.29 (1 H, br s) (Found: M^+, 452.1897. $C_{27}{}^{13}CH_{25}N_3O_3$ requires *M*, 452.1929).

Methyl (*Z*)-2-acetamido-4-methyl[4-^{2}H]pent-2-enoate 2e. According to the procedure for the preparation of compound **2d**, the condensation of crude phophorylglycine (10 mmol) with 2-methyl[2-$^2H_2$]propanal (20 mmol) gave *title compound* **2e** (1.134 g, 61% from *N*-acetylglycine), mp 67–68 °C (from hexane–AcOEt); $\delta_H(CDCl_3)$ 1.05 (6 H, s), 2.12 (3 H, s), 3.77 (3 H, s), 6.53 (1 H, s) and 6.65 (1 H, br s) (Found: M^+, 186.1146. $C_9H_{14}{}^2HNO_3$ requires *M*, 186.1115).

A similar reaction starting from crude methyl *N*-acetyl-2-(dimethoxyphosphoryl)[1-^{13}C]glycinate (13.59 mmol) yielded compound [1-^{13}C]-**2e** (1.04 g, 41% from *N*-acetyl[1-^{13}C]glycine); $\delta_H(CDCl_3)$ 1.04 (6 H, s), 2.12 (3 H, s), 3.76 (3 H, d, *J* 3.7), 6.52 (1 H, d, *J* 4.5) and 6.67 (1 H, br s) (Found: M^+, 187.1160. $C_9{}^{13}CH_{14}{}^2HNO_3$ requires *M*, 187.1148).

Catalytic deuteriation of dehydroamino acids with Pd/C

(*Z*)-2-Acetamido-3-phenylpropenoic acid **1a** (1.00 g, 4.88 mmol) was deuteriated by using D_2 gas (5 kg cm^{-2}) and 10% Pd/C (0.20 g, 20 w/w%) in MeOD (20 cm^3) for 20 h. After removal of the catalyst on a Celite pad, the solution was evaporated to give DL-threo-N-*acetyl*-[2,3-$^2H_2$]*phenylalanine* **3a** (0.894 g, 88%) as an oil, which solidified upon storage. The diastereoselectivity was determined by ^{1}H NMR integration of the β-proton region; $\delta_H[(CD_3)_2SO]$ 1.77 (3 H, s), 2.81 (0.99 H, s), 3.01 (0.01 H, s) and 7.18–7.30 (5 H, m) (Found: M^+, 209.1005. $C_{11}H_{11}{}^2H_2NO_3$ requires *M*, 209.1021).

Other dehydroamino acids **1b**, **2d**, [1-^{13}C]-**1a**, [1-^{13}C]-**1b** and [1-^{13}C]-**2d** were similarly deuteriated. In the case of acids **2d** and [1-^{13}C]-**2d**, isolation of the product from the concentrated reaction mixture was carried out by extraction with water in order to separate it from triphenylmethanol.

DL-threo-N-*Acetyl*[2,3-$^2H_2$]*tyrosine* **3b**; $\delta_H[(CD_3)_2SO]$ 1.78 (3 H, s), 2.68 (>0.99 H, s), 2.87 (<0.01 H, s), 6.65 and 7.00 (4 H, AA′BB′q, *J* 8.5), 8.11 (1 H, s), 9.22 (1 H, br s) (Found: M^+, 225.0997. $C_{11}H_{11}{}^2H_2NO_4$ requires *M*, 225.0970).

DL-threo-N-*Acetyl*[2,3-$^2H_2$]*histidine methyl ester* **4d**; $\delta_H[(CD_3)_2SO]$ 1.81 (3 H, s), 2.80 (0.98 H, s), 2.87 (0.02 H, s), 3.58 (3 H, s), 6.79 (1 H, s) and 8.24 (1 H, s) (Found: M^+, 213.1073. $C_9H_{11}{}^2H_2N_3O_3$ requires *M*, 213.1082).

[1-^{13}C]-**3a**; $\delta_H[(CD_3)_2SO]$ 1.78 (3 H, s), 2.81 (0.99 H, d, *J* 2.9), 3.01 (0.01 H, br d) and 7.18–7.30 (5 H, m) (Found: M^+, 210.1035. $C_{10}{}^{13}CH_{11}{}^2H_2NO_3$ requires *M*, 210.1055).

[1-^{13}C]-**3b**; $\delta_H[(CD_3)_2SO]$ 1.78 (3 H, s), 2.68 (0.98 H, d, *J* 3.1), 2.87 (0.02 H, br d) and 6.65 and 7.00 (4 H, AA′BB′q, *J* 8.5), 8.11 (1 H, s) and 9.22 (1 H, br s) (Found: M^+, 226.1008. $C_{10}{}^{13}CH_{11}{}^2H_2NO_4$ requires *M*, 226.1004).

[1-^{13}C]-**4d**; $\delta_H[(CD_3)_2SO]$ 1.82 (3 H, s), 2.80 (0.97 H, d, *J* 3.4), 2.87 (0.03 H, br d), 3.58 (3 H, d, *J* 3.8), 6.79 (1 H, s) and 8.25 (1 H, s) (Found: M^+, 214.1126. $C_8{}^{13}CH_{11}{}^2H_2N_3O_3$ requires *M*, 214.1116).

DL-*threo*-*N*-Acetyl[2,3-$^2H_2$]tryptophan 3c

To a solution of (*Z*)-2-acetamido-3-(indol-3′-yl)propenoic acid **1c** (1.22 g, 5 mmol) in MeOD (50 cm^3) was added a suspension of $RhCl(PPh_3)_3$ (92.5 mg, 0.1 mmol) in benzene (10 cm^3), and the mixture was deuteriated as described above for 48 h. The mixture was concentrated under reduced pressure and extracted with 0.5 mol dm^{-3} aq. NaOH (20 cm^3). After removal of the insoluble materials using Celite pad, the filtrate was acidified with conc. HCl to afford *title compound* **3c** (1.02 g, 82%) as solid; $\delta_H[(CD_3)_2SO]$ 1.80 (3 H, s), 2.96 (0.98 H, s), 3.12 (0.02 H, s), 6.98 (1 H, m), 7.06 (1 H, m), 7.14 (1 H, d, *J* 2.3), 7.33 (1 H, d, *J* 8.1), 7.52 (1 H, d, *J* 7.8), 8.16 (1 H, s), 10.85 (1 H, s) and 12.60 (1 H, br s) (Found: M^+, 248.1140. $C_{13}H_{12}{}^2H_2N_2O_3$ requires *M*, 248.1130).

Acid [1-^{13}C]-**1c** was similarly deuteriated to give *compound* [1-^{13}C]-**3c**; $\delta_H[(CD_3)_2SO]$ 1.80 (3 H, s), 2.96 (0.98 H, d, *J* 3.0), 3.12 (0.02 H, br d), 6.98 (1 H, m), 7.06 (1 H, m), 7.14 (1 H, d, *J* 2.3), 7.33 (1 H, d, *J* 8.1) 7.52 (1 H, d, *J* 7.8) 8.16 (1 H, s), 10.85 (1 H, s) and 12.61 (1 H, br s) (Found: M^+, 249.1157. $C_{12}{}^{13}CH_{12}{}^2H_2N_2O_3$ requires *M*, 249.1164).

Methyl DL-*threo*-*N*-acetyl[2,3,4-$^2H_3$]leucinate 4e. Methyl (*Z*)-2-acetamido-4-methyl[4-^{2}H]pent-2-enoate **2e** (0.20 g, 1.08 mmol) was deuteriated in an identical manner to that of acid **1c**. Extraction of the concentrated reaction mixture with MeOH–water (1:1) followed by evaporation gave *title compound* **4e** (0.20 g, 98%); $\delta_H[(CD_3)_2SO]$ 0.83 (3 H, s), 0.88 (3 H, s), 1.48 (1 H, s), 1.84 (3 H, s), 3.61 (3 H, s) and 8.22 (1 H, br s) (Found: M^+, 190.1394. $C_9H_{14}{}^2H_3NO_3$ requires *M*, 190.1397).

Compound [1-^{13}C]-**4e** was prepared from compound [1-^{13}C]-**2e** in an identical manner to that used for the unlabelled compound above; $\delta_H[(CD_3)_2SO]$ 0.83 (3 H, s), 0.88 (3 H, s), 1.48 (1 H, d, *J* 3.3), 1.84 (3 H, s), 3.61 (3 H, d, *J* 3.8) and 8.23 (1

H, br s) (Found: M$^+$, 191.1411. $C_8{}^{13}CH_{14}{}^2H_3N_2O_3$ requires *M*, 191.1430).

Optical resolution of acetamido acid derivatives 3 and isolation of L-*threo*- and L-*erythro*-[2,3-$^2H_2$] amino acids 5

DL-*threo*-*N*-Acetyl[1-^{13}C, 2,3-$^2H_2$]phenylalanine **3a** (1.02 g, 4.85 mmol) and $CoCl_2$·$6H_2O$ (12 mg, 0.05 mmol) were dissolved in 2.5 mol dm^{-3} aq. NaOH (15 cm^3) and the pH was adjusted to 8.0–8.5 (pH test paper) using 1 mol dm^{-3} HCl. The solution was treated with crude, powdered *Aspergillus* acylase (30 mg, 30 w/w%) and was incubated at 37 °C for 48–72 h. The reaction mixture was concentrated to dryness and the residue was dissolved in 1 mol dm^{-3} HCl. The insoluble material, mainly unchanged D-*threo*-[1-^{13}C]-**3a**, was collected and the filtrate was submitted to ion-exchange column chromatography on DOWEX 50W-X8 and the resin was washed with water (500 cm^3). D-*threo*-[1-^{13}C]-**3a** was also recovered from the aqueous washings. Elution with 1 mol dm^{-3} aq. NH_4OH and evaporation of appropriate fractions (monitored by ninhydrin spray) gave L-threo-[1-^{13}C, 2,3-$^2H_2$]*phenylalanine* **5a** (347 mg, 85%). The de was determined by ^{1}H NMR spectroscopy and the optical purity (100%) was checked by HPLC using a chiral column; $\delta_H(D_2O)$ 3.09 (0.98 H, br d), 3.25 (0.02 H, br d), 7.29–7.44 (m, 5 H); $\delta_C(D_2O$; ^{1}H coupled) 173.7 (d, *J* 2.6) (Found: M$^+$, 168.0962. $C_8{}^{13}CH_9{}^2H_2NO_2$ requires *M*, 168.0949).

The recovered acetamido acid D-*threo*-[1-^{13}C]-**3a** was racemized using the modified method of Wood and Vigneaud.[22] Thus, to a solution of compound D-*threo*-[1-^{13}C]-**3a** (2.43 mmol) in 2.5 mol dm^{-3} aq. NaOD (2 cm^3) was added acetic anhydride (0.50 g, 4.85 mmol) dropwise over a period of 1 h at 70 °C under argon. The progress of the racemization was monitored by ^{1}H NMR spectroscopy. After additional heating for 30 min, the reaction mixture was evaporated and the residue containing D-*threo*-[1-^{13}C]-**3a** and L-*erythro*-[1-^{13}C]-**3a** was directly subjected to optical resolution as described above to give L-erythro-[1-^{13}C 2,3-$^2H_2$]*phenylalanine* **5a** (140 mg, 69%). The optical purity was also checked by HPLC (100%); $\delta_H(D_2O)$ 3.09 (0.03 H, br s), 3.25 (0.97 H, d, *J* 3.8) and 7.29–7.44 (5 H, m); $\delta_C(D_2O$; ^{1}H coupled) 173.7 (d, *J* 3.8) (Found: M$^+$, 168.0925. $C_8{}^{13}CH_9{}^2H_2NO_2$ requires *M*, 168.0949). The unreactive D-*threo*-[1-^{13}C]-**3a** was again recovered. ^{1}H and ^{13}C NMR spectra were identical with those of the DL-form.

Other DL-*threo*-acetamido acid derivatives **3** were similarly treated to afford the corresponding L-*threo*- and L-*erythro*-amino acids **5**. In the case of compounds **4e** and [1-^{13}C]-**4e**, the ester group was hydrolysed using 2.5 mol dm^{-3} NaOD (2–3 mol equiv.) in MeOD prior to the optical resolution.

L-threo-[2,3-$^2H_2$]*Phenylalanine* **5a**; $\delta_H(D_2O)$ 3.09 (0.99 H, s), 3.25 (0.01 H, s) and 7.25–7.45 (5 H, m) (Found: M$^+$, 167.0905. $C_9H_9{}^2H_2NO_2$ requires *M*, 167.0915).

L-erythro-[2,3-$^2H_2$]*Phenylalanine* **5a**; $\delta_H(D_2O)$ 3.09 (0.02 H, s), 3.25 (0.98 H, s) and 7.29–7.44 (5 H, m) (Found: M$^+$, 167.0916).

L-threo-[2,3-$^2H_2$]*Tyrosine* **5b**; $\delta_H(D_2O)$ 2.64 (0.99 H, s), 2.82 (0.01 H, s) and 6.57 and 6.98 (4 H, AA'BB'q, *J* 8.4) (Found: M$^+$, 183.0861. $C_9H_9{}^2H_2NO_3$ requires *M*, 183.0864).

L-erythro-[2,3-$^2H_2$]*Tyrosine* **5b**; $\delta_H(D_2O)$ 2.64 (0.05 H, s), 2.82 (0.95 H, s) and 6.57 and 6.98 (4 H, AA'BB'q, *J* 8.4) (Found: M$^+$, 183.0870).

L-threo-[1-^{13}C, 2,3-$^2H_2$]*Tyrosine* **5b**; $\delta_H(D_2O)$ 2.64 (0.97 H, d, *J* 2.4), 2.82 (0.03 H, br d) and 6.57 and 6.98 (4 H, AA'BB'q, *J* 8.4); $\delta_C(D_2O$; ^{1}H coupled) 181.1 (d, *J* 2.4) (Found: M$^+$, 184.0889. $C_8{}^{13}CH_9{}^2H_2NO_3$ requires *M*, 184.0898).

L-erythro-[1-^{13}C, 2,3-$^2H_2$]*Tyrosine* **5b**; $\delta_H(D_2O)$ 2.64 (0.06 H, br d), 2.82 (0.94 H, d, *J* 4.0) and 6.57 and 6.98 (4 H, AA'BB'q, *J* 8.4); $\delta_C(D_2O$; ^{1}H coupled) 181.1 (d, *J* 4.0) (Found: M$^+$, 184.0881).

L-threo-[2,3-$^2H_2$]*Tryptophan* **5c**; $\delta_H(D_2O)$ 3.28 (0.98 H, s), 3.45 (0.02 H, s), 7.19 (1 H, m), 7.27 (1 H, m), 7.31 (1 H, s), 7.53 (1 H, d, *J* 8.2) and 7.72 (1 H, d, *J* 7.9) (Found: M$^+$, 206.1035. $C_{11}H_{10}{}^2H_2N_2O_2$ requires *M*, 206.1024).

L-erythro-[2,3-$^2H_2$]*Tryptophan* **5c**; $\delta_H(D_2O)$ 3.28 (0.07 H, s), 3.45 (0.93 H, s), 7.18 (1 H, m), 7.27 (1 H, m), 7.29 (1 H, s), 7.52 (1 H, d, *J* 8.2) and 7.73 (1 H, d, *J* 7.9) (Found: M$^+$, 206.1059).

L-threo-[1-^{13}C, 2,3-$^2H_2$]*Tryptophan* **5c**; $\delta_H(D_2O)$ 3.24 (0.95 H, br d), 3.41 (0.05 H, br d), 7.18 (1 H, m), 7.27 (1 H, m), 7.29 (1 H, s), 7.52 (1 H, d, *J* 8.2) and 7.72 (1 H, d, *J* 7.9); $\delta_C(D_2O$; ^{1}H coupled) 175.2 (d, *J* 1.52) (Found: M$^+$, 207.1078. $C_{10}{}^{13}CH_{10}{}^2H_2N_2O_2$ requires *M*, 207.1058).

L-erythro-[1-^{13}C, 2,3-$^2H_2$]*Tryptophan* **5c**; $\delta_H(D_2O)$ 3.22 (0.11 H, br d), 3.39 (0.89 H, d, *J* 3.9), 7.18 (1 H, m), 7.27 (1 H, m), 7.29 (1 H, s), 7.52 (1 H, d, *J* 8.2) and 7.72 (1 H, d, *J* 7.9); $\delta_C(D_2O$; ^{1}H coupled) 176.1 (d, *J* 3.9) (Found: M$^+$, 207.1058).

L-threo-[2,3,4-$^2H_3$]*Leucine* **5e**; $\delta_H(D_2O)$ 0.93 (3 H, s), 0.95 (3 H, s), 1.64 (0.99 H, s) and 1.70 (0.01 H, s) (Found: M$^+$, 135.1232. $C_6H_{11}{}^2H_3NO_2$ requires *M*, 135.1213).

L-erythro-[2,3,4-$^2H_3$]*Leucine* **5e**; $\delta_H(D_2O)$ 0.93 (3 H, s), 0.95 (3 H, s), 1.64 (0.07 H, s) and 1.70 (0.93 H, s) (Found: M$^+$, 135.1257).

L-threo-[1-^{13}C, 2,3,4-$^2H_3$]*Leucine* **5e**; $\delta_H(D_2O)$ 0.93 (3 H, s), 0.95 (3 H, s), 1.64 (0.96 H, br d) and 1.70 (0.04 H, br d); $\delta_C(D_2O$; ^{1}H coupled) 175.1 (d, *J* 3.1) (Found: M$^+$, 136.1246. $C_5{}^{13}CH_{11}{}^2H_3NO_2$ requires *M*, 136.1246).

L-erythro-[1-^{13}C, 2,3,4-$^2H_3$]*Leucine* **5e**; $\delta_H(D_2O)$ 0.93 (3 H, s), 0.95 (3 H, s), 1.64 (0.08 H, br d) and 1.70 (0.92 H, br d); $\delta_C(D_2O$; ^{1}H coupled) 175.1 (d, *J* 3.0) (Found: M$^+$, 136.1281).

Isolation of L-*threo*- and L-*erythro*-[2,3-$^2H_2$]histidine 5d

Methyl DL-*threo*-*N*-acetyl-[2,3-$^2H_2$]histidinate **4d** (1.49 g, 7.00 mmol) was hydrolysed using 2.5 mol dm^{-3} NaOD (8.4 cm^3) in MeOD (14 cm^3) prior to the optical resolution. After evaporation of the solvent, the residue was dissolved in water (50 cm^3) and the pH was adjusted to 7.0–7.5 (pH test paper) using 1 mol dm^{-3} HCl. To the solution was added powdered porcine kidney acylase (40 mg) and the mixture was incubated at 37 °C for 48–72 h. The reaction mixture was directly submitted to ion-exchange column chromatography on Amberlite IRC-50 and the resin was washed with water (1200 cm^3). To complete the hydrolysis of the L-form, the concentrated aqueous washings which contained D-*threo*-**3d** and a small amount of unchanged L-*threo*-**3d** were treated several times (2–3 times) with the acylase as described above. Elution with 1 mol dm^{-3} HCl, evaporation, and chromatography on DOWEX 50W-X8 gave L-threo-[2,3-$^2H_2$]*histidine* **5d** (545 mg, 99%). The de was determined by ^{1}H NMR spectroscopy and the optical purity (100%) was checked by HPLC on a chiral column; $\delta_H(D_2O)$ 3.08 (0.97 H, s), 3.18 (0.03 H, s), 7.03 (1 H, s) and 7.73 (1 H, s) (Found: M$^+$, 157.0822. $C_6H_7{}^2H_2N_3O_3$ requires *M*, 157.0820).

Racemization of the recovered substrate D-*threo*-**3d** was carried out by deacetylation followed by acetylation because the procedure mentioned previously was not so effective for compound **3d**. Thus, compound D-*threo*-**3d** (3.5 mmol) was hydrolysed in refluxing 6 mol dm^{-3} DCl (15 cm^3) for 2 h. After evaporation of the solvent, the residue was dissolved in 2.5 mol dm^{-3} aq. NaOD (4.2 cm^3), and acetic anhydride (7.14 g, 70 mmol) was added dropwise over a period of 1 h at room temp. under argon. After being stirred at 70 °C overnight, the reaction mixture was evaporated and the residue containing epimers D-*threo*-**3a** and L-*erythro*-**3a** was directly subjected to optical resolution as described above to give L-erythro-[2,3-$^2H_2$]*histidine* **5d** (64 mg, 23%). The optical purity was also checked by HPLC (100%); $\delta_H(D_2O)$ 3.10 (0.06 H, s), 3.20 (0.94 H, s), 7.06 (1 H, s) and 7.82 (1 H, s) (Found: M$^+$ 157.0827. $C_6H_7{}^2H_2N_3O_3$ requires *M*, 157.0820).

The 1-^{13}C derivative of compound **4d** was similarly treated to

afford L-*threo*-[1-^{13}C, 2,3-$^{2}H_2$]histidine **5d** and L-*erythro*-[1-^{13}C]-**5d**.

L-threo-[1-^{13}C, 2,3-$^{2}H_2$]*Histidine* **5d**; $\delta_H(D_2O)$ 3.08 (0.97 H, br s), 3.18 (0.03 H, br d), 7.03 (1 H, s) and 7.73 (1 H, s); $\delta_C(D_2O$; ^{1}H coupled) 174.0 (d, *J* 2.3) (Found: M^+, 158.0857. $C_5{}^{13}CH_7{}^2H_2N_3O_3$ requires *M*, 158.0854).

L-erythro-[1-^{13}C, 2,3-$^{2}H_2$]*Histidine* **5d**; $\delta_H(D_2O)$ 3.09 (0.08 H, br s), 3.19 (0.92 H, br d, *J* 3.8), 7.03 (1 H, s) and 7.73 (1 H, s); $\delta_C(D_2O$; ^{1}H coupled) 174.8 (d, *J* 3.8) (Found: M^+, 158.0867).

Acknowledgements

We thank The Science and Technology Agency of the Japanese Government for partial financial support through the Special Coordination Funds.

References

1 K. Wuthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, 1986; G. C. K. Roberts, *NMR of Macromolecules. A Practical Approach*, Oxford University Press, 1993.
2 M. Kainosho and K. Ajisaka, *J. Am. Chem. Soc.*, 1975, **97**, 5630.
3 D. Cowburn, D. H. Live, A. J. Fischman and W. C. Agosta, *J. Am. Chem. Soc.*, 1983, **105**, 7435; A. J. Fischman, D. H. Live, H. R. Wyssbrod, W. C. Agosta and D. Cowburn, *J. Am. Chem. Soc.*, 1980, **102**, 2533.
4 H. Kessler, C. Griesinger and K. Wagner, *J. Am. Chem. Soc.*, 1987, **109**, 6927.
5 J. Kobayashi and U. Nagai, *Tetrahedron Lett.*, 1977, 1803; J. Kobayashi, T. Higashijima and T. Miyazawa, *Int. J. Pept. Protein Res.*, 1984, **24**, 40.
6 K. Nishiyama, M. Oba, R. Ueno, A. Morita, Y. Nakamura and M. Kainosho, *J. Labelled Compd. Radiopharm.*, 1994, **34**, 831.
7 K. G. R. Pachler, *Spectrochim. Acta*, 1964, **20**, 581.
8 H. E. Carter, in *Org. React.* 1959, **3**, 198.
9 M. Oba, Y. Kawahara, K. Nishiyama, M. Kitsukawa and M. Kainosho, *Bull. Sch. High-Tech. Tokai Univ.*, 1993, **3**, 125.
10 U. Schmidt, A. Lieberknecht and J. Wild, *Synthesis*, 1984, 53.
11 R. Kober and W. Steglich, *Liebigs Ann. Chem.*, 1983, 599.
12 U. Schmidt, H. Griesser, V. Leitenberger, A. Lieberknecht, R. Mangold, R. Meyer and B. Riedl, *Synthesis*, 1992, 487.
13 J. L. Kelley, C. A. Miller and E. W. McLean, *J. Med. Chem.*, 1977, **20**, 721.
14 J. A. Osborn and G. Wilkinson, *Inorg. Synth.*, 1967, **10**, 67.
15 P. S. Hallman, T. A. Stephenson and G. Wilkinson, *Inorg. Synth.*, 1970, **12**, 237.
16 R. M. Herbst and D. Schemin, *Org. Synth.* 1943, **2**, 1.
17 H. B. Gillespie and H. R. Snyder, *Org. Synth.* 1943, **2**, 489.
18 H. D. Dakin, *J. Biol. Chem.*, 1932, **82**, 443.
19 E. Erlenmeyer and J. T. Halsey, *Justus Liebig's Ann. Chem.*, 1899, **307**, 138.
20 A. Ellinger and Flamand, *Ber. Dtsch. Chem. Ges.*, 1907, **40**, 3029.
21 F. L. Pyman, *J. Chem. Soc.*, 1916, **109**, 186.
22 J. L. Wood and V. du Vigneaud, *J. Biol. Chem.*, 1939, **130**, 109.

Paper 4/07034H
Received 17*th November* 1994
Accepted 27*th January* 1995