Syntheses of isotopically labelled L-α-amino acids with an asymmetric centre at C-3

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Approaches are described to the synthesis of a series of isotopically labelled L-α-amino acids each with an asymmetric centre at C-3, including isoleucine, *allo*-isoleucine, threonine and *allo*-threonine. The methods may be simply adapted for the selective incorporation of an isotopic label at each site of L-valine including the selective labelling of either diastereotopic methyl group with carbon-13 and/or deuterium and labelling of the amine with nitrogen-15.

Introduction

The hydrophobic side-chains of L-amino acids such as leucine, valine and isoleucine are important to the three-dimensional structure of proteins. The incorporation of these amino acids selectively labelled with carbon-13, deuterium and nitrogen-15 into proteins, combined with heteronuclear NMR experiments, facilitates the assignments of the complex ^{1}H-NMR resonances of proteins and thus enables the elucidation of their three-dimensional structures.[1] In addition, amino acids incorporating stable isotopic labels are valuable for metabolic studies.[2] Thus, the design of efficient strategies for the synthesis of amino acids selectively labelled with stable isotopes is a rapidly expanding field.[3] The selective isotopic labelling of isoleucine and *allo*-isoleucine is further complicated as the stereochemistry of two asymmetric centres needs to be controlled. Previous syntheses of these compounds include the reaction of allylic boron compounds with α-imino esters,[4] inversion of configuration of L-isoleucine to D-*allo*-isoleucine[5] and a racemic synthesis of isotopically labelled material was reported by Parry *et al.*[6] Chamberlin and co-workers[7] have developed an efficient method for the preparation of L-isoleucine with carbon-13 in the 3-methyl group starting with L-aspartic acid and using $^{13}CH_3I$ as the source of isotopic label. More recently we have described a chemo-enzymatic route for the synthesis of L-isoleucine and L-*allo*-isoleucine which enabled the selective incorporation of deuterium and carbon-13 into the 3-methyl group as well as the inclusion of nitrogen-15.[8] Our strategy involved use of chiral enolates derived from Evans' oxazolidinone imides[9] to introduce an isotopic label into the 3-methyl group as well as to create the stereogenic centre which was to become C-3 in the target compounds. Cleavage of the auxiliary followed by a one-carbon homologation of the resultant carboxylic acid gave the required 2-keto ester and then biotransformations were used to hydrolyse the ester and to catalyse the reductive amination of the resultant 2-keto acid to establish the stereogenic centre at C-2 of the target α-amino acid. We now describe this approach in full, as well as an adaptation of this methodology to prepare L-threonine and L-*allo*-threonine,[10] and our more recent investigations which have led to methods for the selective introduction of an isotopic label into each site of L-valine.

Results and discussion

Syntheses of L-isoleucine, *allo*-isoleucine, threonine and *allo*-threonine

Our approach to the synthesis of isotopically labelled L-isoleucine is illustrated in Scheme 1. Reaction of **1** with butyllithium followed by butyryl chloride gave the oxazolidinone **2** in quantitative yield.[11] Alkylation of the sodium enolate of **2** with methyl iodide ($^{13}CH_3I$ or CD_3I) gave an 18 : 1 ratio of the oxazolidinone **3** and its diastereoisomer. The chiral auxiliary was then cleaved under standard conditions[12] with lithium hydroxide and hydrogen peroxide to give (*S*)-2-methylbutanoic acid (*S*)-**4**. If no isotopic label is required in this fragment then commercially available (*S*)-2-methylbutanoic acid may be used. Many methods are known for the conversion of carboxylic acids to 2-keto esters,[13] however a method was required which would not cause racemisation α to the carbonyl group and we favoured an approach described by Wasserman and Ho.[14] Thus coupling (cyanomethylene)triphenylphosphorane with (*S*)-2-methylbutanoic acid in the presence of EDCI and DMAP [EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] gave (*S*)-**5** which on ozonolysis furnished the required 2-keto ester (*S*)-**6** in 75% yield over the two steps.

The final stages of the synthesis of L-isoleucine required hydrolysis of the ester and reductive amination of the resultant 2-keto acid catalysed by commercially available leucine dehydrogenase (LeuDH). This enzyme requires the cofactor NADH which may be efficiently recycled *in situ* according to the protocol of Shaked and Whitesides using a second commercially available enzyme formate dehydrogenase (FDH) with the consumption of formate ions and the evolution of carbon dioxide.[15] Previously we have converted 2-keto esters to 2-keto acids with sodium hydroxide,[16] however, in this case, these conditions led to racemisation. A milder method is to use the lipase isolated from *Candida rugosa* (CRL). Since the reaction conditions are compatible with those used in the reductive amination of the 2-keto acid catalysed by leucine dehydrogenase, the ester (*S*)-**6** was directly converted in one-pot to L-isoleucine by a hydrolysis–reductive amination sequence in 74% yield. When [^{15}N]ammonium formate was used in the reductive amination reaction, [^{15}N]isoleucine was obtained with complete stereocontrol at C-2 and with 90% de at C-3.

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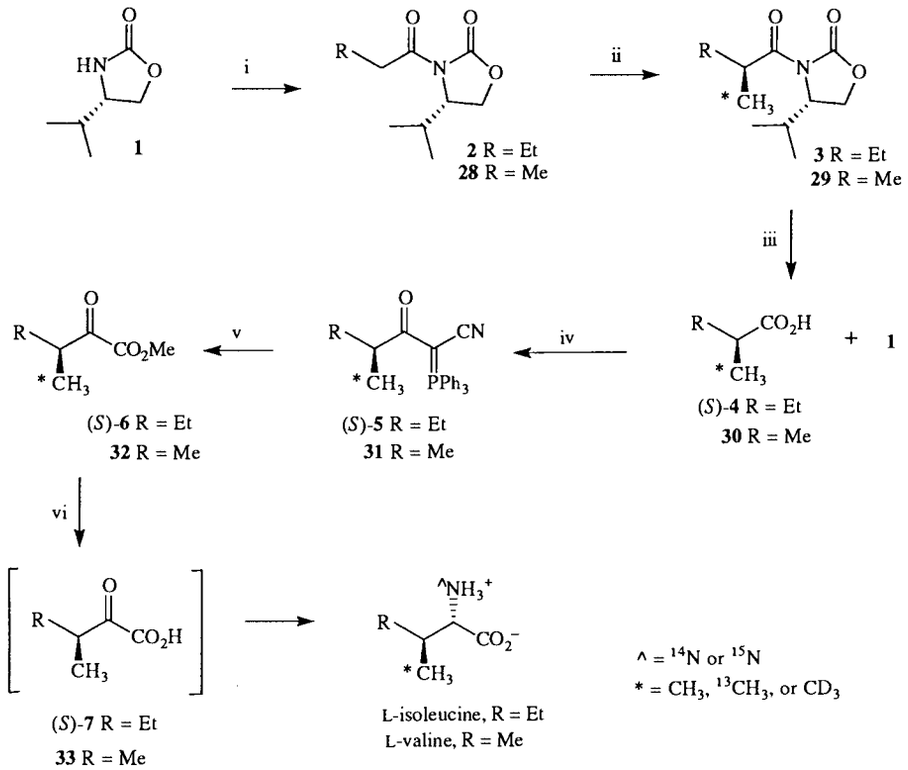
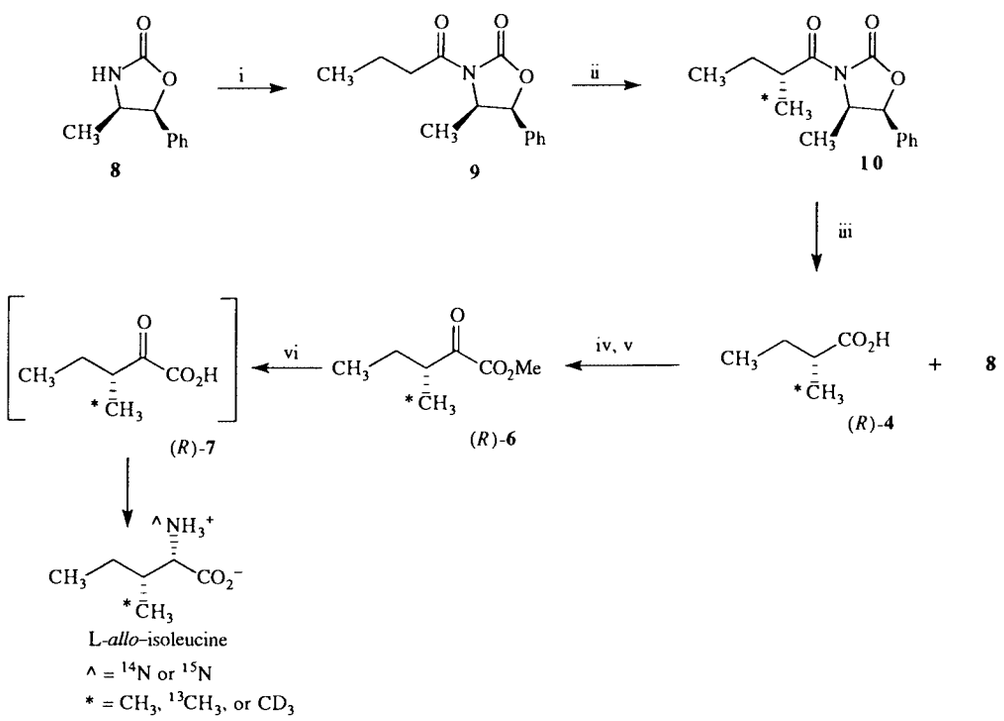


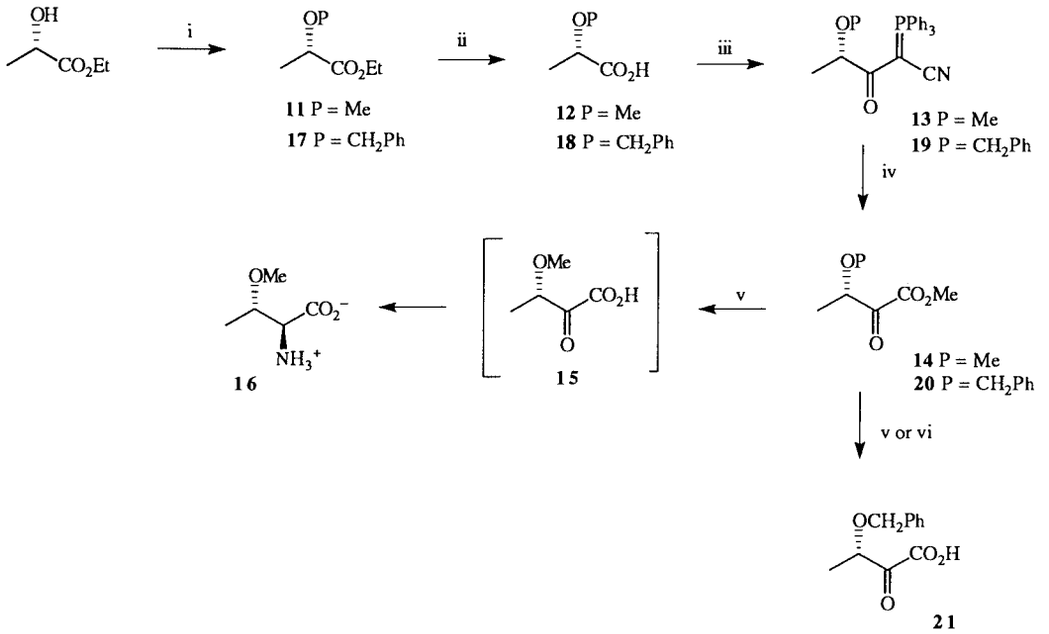
Scheme 1 *Reagents*: i, BuLi, either PrCOCl or EtCOCl; ii, NaHMDS, *CH_3I; iii, LiOH, H_2O_2; iv, Ph_3PCHCN, EDCI, DMAP; v, O_3, MeOH, CH_2Cl_2; vi, CRL then LeuDH, FDH, NADH, $HCO_2{}^{\wedge}NH_4$.



Scheme 2 *Reagents:* i,BuLi, PrCOCl; ii, NaHMDS, *CH_3I; iii, LiOH, H_2O_2; iv, Ph_3PCHCN, EDCI, DMAP; v, O_3, MeOH, CH_2Cl_2; vi, CRL then LeuDH, FDH, NADH, $HCO_2{}^{\wedge}NH_4$.

L-*allo*-[^{15}N]Isoleucine was prepared using an analogous approach to that for L-[^{15}N]isoleucine but starting from the chiral auxiliary[11a] derived from (+)-norephedrine **8** (Scheme 2). Alkylation of the known oxazolidinone **9**[11b] using sodium hexamethyldisilazide and methyl iodide gave a 13:1 ratio of **10** and its diastereomer. Hydrolytic cleavage of the chiral auxiliary gave (*R*)-2-methylbutanoic acid (*R*)-**4** which was converted to the required 2-keto ester (*R*)-**6** in 61% overall yield from **10**. Using [^{15}N]ammonium formate in a one-pot, dual enzymatic hydrolysis and reductive amination procedure, 2-keto ester (*R*)-**6** was converted to L-*allo*-[^{15}N]isoleucine in 74% yield after purification by ion exchange chromatography. To the best of our knowledge this is the first reported synthesis of L-*allo*-[^{15}N]isoleucine and the approach can be readily adapted to introduce carbon-13 and deuterium in the 3-methyl group using the appropriately isotopically labelled methyl iodide as the electrophile in the alkylation reaction.

One potential drawback of this approach as a general method for the synthesis of further isotopically labelled L-α-amino acids with a stereogenic centre at C-3, particularly



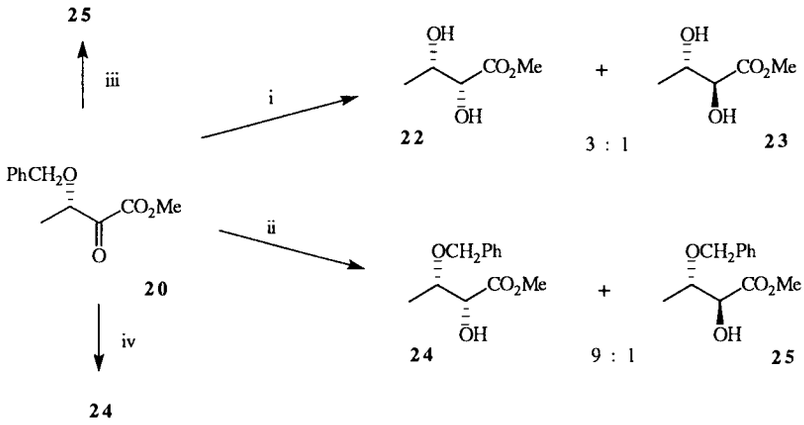
Scheme 3 *Reagents*: i, NaH, MeI or NaH, $PhCH_2Br$; ii, LiOH, MeOH, H_2O; iii, Ph_3PCHCN, EDCI, DMAP; iv, O_3, MeOH, CH_2Cl_2; v, CRL then LeuDH, FDH, NADH, HCO_2NH_4; vi, CRL then PheDH, FDH, NADH, HCO_2NH_4.

those such as threonine and *allo*-threonine containing a heteroatom in the side-chain, is that leucine dehydrogenase and other enzymes of this superfamily have been reported to have limited substrate specificities.[17] However, having shown in the above syntheses of L-isoleucine and *allo*-isoleucine that 3-methyl-2-oxopentanoic acids (*S*)-**7** and (*R*)-**7** are good substrates for leucine dehydrogenase, we reasoned on steric grounds that (*S*)-3-methoxy-2-oxobutanoic acid **15** and the (*R*)-enantiomer should also be substrates for the dehydrogenase giving access, after deprotection, to L-*allo*-[^{15}N]threonine and L-[^{15}N]threonine respectively. Thus methyl (*S*)-3-methoxy-2-oxobutanoate **14** was prepared as shown in Scheme 3. Ethyl (*S*)-lactate was protected as the methyl ether **11** by reaction with methyl iodide and sodium hydride. Hydrolysis of the ester with sodium hydroxide led to epimerisation whereas with lithium hydroxide, the required (*S*)-2-methoxypropanoic acid **12** was obtained in 75% yield with an optical rotation of −68.4 (neat) [lit.[18] $[a]_D$ −70.5 (neat)]. A one-carbon homologation of the acid **12** *via* ozonolysis of β-ketocyanophosphorane **13** gave 2-keto ester **14**. A one-pot enzyme catalysed hydrolysis of the ester and reductive amination of the resultant 2-keto acid **15** gave (2*S*,3*S*)-2-amino-3-methoxybutanoic acid **16** in 73% yield as a single diastereomer. Unfortunately repeated attempts to deprotect the methyl ether in **16** for example with 6 M hydrochloric acid, boron tribromide or boron trifluoride–diethyl ether–propane thiol, failed to give L-*allo*-threonine and simply returned the ether.

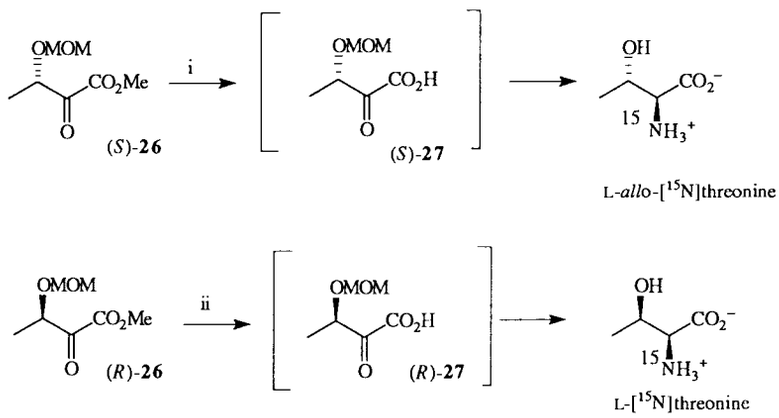
Wasserman has reported[14] that the hydroxy groups in the side-chain of a carboxylic acid must be protected prior to coupling with (cyanomethylene)triphenylphosphorane, therefore an alternative protecting group was required for lactic acid and the use of a benzyl ether was investigated. Methyl (*S*)-3-benzyloxy-2-oxobutanoate **20** was prepared in 4 steps from ethyl (*S*)-lactate *via* the approach shown in Scheme 3 using sodium hydride and benzyl bromide to protect the alcohol. Although the ester **20** was cleanly hydrolysed using *Candida rugosa* lipase, the resultant (*S*)-3-benzyloxy-2-oxobutanoic acid **21** was recovered unchanged when incubated with either leucine dehydrogenase or phenylalanine dehydrogenase (PheDH). These results demonstrate the problem of the relatively narrow substrate specificity range of these enzymes. Therefore, it was apparent that a less bulky group was required at C-3 than the benzyl ether for the synthesis of L-*allo*-[^{15}N]threonine. It has been shown that 3-hydroxypyruvate is readily converted to L-serine using amino acid dehydrogenases to catalyse the reductive amination.[19] By analogy, we reasoned that if we could prepare 3-hydroxy-2-oxobutanoic acid that this may also be a substrate for these enzymes. With this in mind, the benzyl ether **20** was stirred under an atmosphere of hydrogen in the presence of a palladium on calcium carbonate catalyst, but although this led to the required hydrogenolysis reaction, it was accompanied by reduction of the ketone giving a 3 : 1 mixture of the known[20] dihydroxy esters **22** and **23** (Scheme 4). This result was not too surprising as Mitsui and co-workers have shown that carbonyl compounds with adjacent electron withdrawing groups are activated towards catalytic reduction and there are many examples of catalytic reduction of 2-keto esters using catalysts such as PtO_2, Pd/C and $Pd/CaCO_3$.[21] The stereochemical outcome of the reduction of 2-keto ester **20** with a range of reducing agents was examined and although $NaBH_4$ and $Na(CN)BH_3$ gave approximately a 1 : 1 mixture of **24** and **25**, improved diastereoselection was achieved using L-selectride giving a 9 : 1 mixture in favour of **24**.

In order to compare the substrate specificities of the amino acid dehydrogenases (LeuDH and PheDH) with a further oxidoreductase, L-lactate dehydrogenase (L-LDH) and to give an approach to the enantioselective synthesis of 3-benzyloxy-2-hydroxybutanoates, methyl (*S*)-3-benzyloxy-2-oxobutanoate **20** was hydrolysed with *Candida rugosa* lipase and then incubated with commercially available LDH from *Bacillus stearothermophilus* (*BS*-LDH).[22] Like the amino acid dehydrogenases, this enzyme requires the cofactor NADH which was readily recycled using formate dehydrogenase.[15] Following reduction of **21**, the resultant hydroxy acid was methylated with etheral diazomethane giving the (*S*)-2-hydroxy ester **24** in 96% overall yield. The (*R*)-2-hydroxy ester **25** was readily prepared in 95% yield using D-hydroxyisocaproate dehydrogenase from *Lactobacillus delbruekeii* (LB-hicDH) to catalyse the reduction of **21**.[23] Thus, unlike the amino acid dehydrogenase catalysed reductive amination of **21**, the simple reduction of the keto acid catalysed by either *BS*-LDH or *LB*-hicDH proceeded in excellent yield and with complete stereocontrol giving access to **24** and **25**, useful building blocks for the preparation of more complex molecules.

Therefore for the preparation of L-*allo*-threonine it was apparent that a protecting group was required for methyl (*S*)-3-hydroxy-2-oxobutanoic acid which would permit the enzyme catalysed reductive amination step and also be more labile than



Scheme 4 *Reagents*: i, H_2, Pd on $CaCO_3$; ii, L-selectride, THF; iii, CRL then *BS*-LDH, FDH, NADH then CH_2N_2; iv, CRL then *LB*-hicDH, FDH, NADH, then CH_2N_2.



Scheme 5 *Reagents*: i, CRL then LeuDH, FDH, NADH, $HCO_2{}^{15}NH_4$ then 2 M HCl; ii, CRL then PheDH, FDH, NADH, $HCO_2{}^{15}NH_4$ then 2 M HCl.

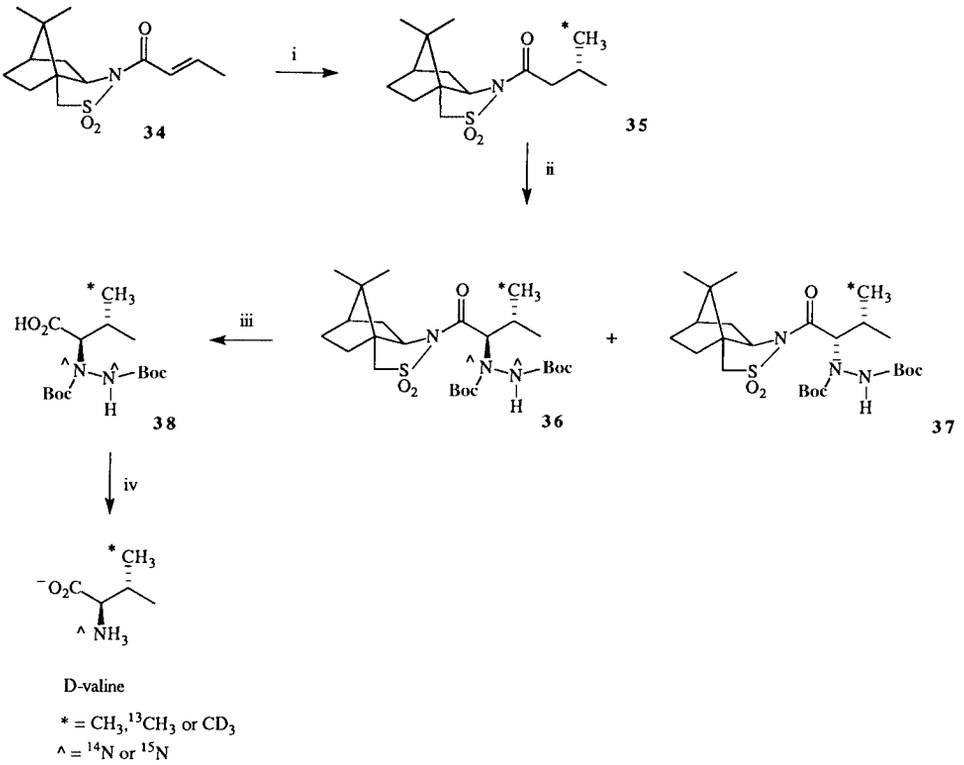
the methyl ether protecting group to deprotection to the amino acid and the methoxymethoxy ether (MOM) proved ideal (Scheme 5). Methyl (*S*)-3-methoxymethoxy-2-oxobutanoate (*S*)-**26** was prepared from ethyl (*S*)-lactate as previously described.[24] Using a one-pot dual enzyme catalysed hydrolysis and reductive amination procedure in the presence of [^{15}N]-ammonium formate and removal of the MOM protecting group with 2 M HCl gave L-*allo*-[^{15}N]threonine in 93% isolated yield after ion exchange chromatography from 2-keto ester (*S*)-**26**. Only a single diastereomer was apparent by both ^{1}H- and ^{13}C-NMR spectroscopy.

A similar strategy was adopted for the synthesis of L-[^{15}N]-threonine from methyl (*R*)-lactate. However the results from the biotransformations with methyl (*R*)-3-methoxymethoxy-2-oxobutanoate (*R*)-**26** were most disappointing: on incubation of (*R*)-**26** with *Candida rugosa* lipase and leucine dehydrogenase only 8% yield of the required amino acid was obtained. Thus, it was apparent that the (*S*)-enantiomer of 2-keto acid (*S*)-**27** was a much better substrate for leucine dehydrogenase than the (*R*)-enantiomer and indeed on incubation of racemic **27** with leucine dehydrogenase followed by acid-catalysed deprotection of the MOM group a 4:1 mixture of L-*allo*-threonine: L-threonine was obtained indicating that a combined kinetic resolution and reduction was observed. This novel kinetic resolution–reductive amination reaction enables the creation of two asymmetric centres in one step and the side-chain interactions in the active site of leucine dehydrogenase are being further investigated.[25] To overcome the problem, phenylalanine dehydrogenase was used to catalyse the reductive amination step and when the reaction was carried out in the presence of [^{15}N]ammonium formate, 2-keto ester (*R*)-**26** was converted cleanly to L-[^{15}N]threonine in 61% yield after ion exchange chromatography.

Two approaches to the syntheses of isotopically labelled L-valine

Assignment of the *pro-R* and *pro-S* methyl groups of valine residues in the NMR spectra of proteins would allow the three dimensional structures of proteins to be more precisely defined. In addition, the selective labelling of either diastereotopic methyl group of L-valine has enabled the elucidation of their fate during the biosynthesis of natural products.[26] Thus, several groups have investigated approaches to the synthesis of valine selectively labelled in either diastereotopic methyl group for example *via* biosynthesis and using chemical and enzymatic resolution.[3,27] In 1995, the groups of Baldwin[28] and Chamberlin[7] demonstrated the use of L-aspartic acid as a starting material for the synthesis of L-valine, selectively labelled in one of the diastereotopic methyl groups by two different strategies. Our approach to the synthesis of L-isoleucine described above may be readily adapted for the preparation of valine selectively labelled with deuterium or carbon-13 in either diastereotopic methyl group as shown in Scheme 1.

Alkylation of the propionylated oxazolidinone **28** with either CD_3I or $^{13}CH_3I$ gave a 13:1 mixture of **29** and its diastereomer as previously described.[29] The oxazolidinone was cleaved with lithium hydroxide and hydrogen peroxide to give (*R*)-2-methyl[3,3,3-D_3]propanoic acid **30** in 78% yield. Treatment of **30** with (cyanomethylene)triphenylphosphorane in the presence of EDCI and DMAP gave the β-ketocyanophosphorane **31** which on oxidative cleavage with ozone in MeOH–CH_2Cl_2 gave methyl (*R*)-3-methyl-2-oxo[4,4,4-D_3]butanoate **32** in 63% yield over the two steps. 2-Keto ester **32** was converted directly in one pot to (2*S*,3*R*)-[4,4,4-D_3]valine in 80% yield after purification by ion exchange chromatography using *Candida rugosa* lipase to catalyse the hydrolysis of the ester and leucine dehydrogenase for the reductive amination of the resultant



Scheme 6 *Reagents*: i, $(*CH_3)_2CuLi{\cdot}PBu_3$; ii, NaHMDS, DBAD; iii, LiOH, H_2O_2; iv, TFA, CH_2Cl_2 then PtO_2, H_2, 35 psi, H_2O.

2-keto acid **33**. The ^{1}H-NMR spectrum of L-valine showed doublets (*J* 7 Hz) at δ 0.94 and 0.89 assigned to the *pro-R* and *pro-S* methyl groups whereas for (2*S*,3*R*)-[4,4,4-D_3]valine only the doublet (at δ 0.94) was apparent confirming that no racemisation had occurred at C-3. Adding [^{15}N]ammonium formate to the enzyme catalysed reaction gave (2*S*,3*R*)-[^{15}N,4,4,4-D_3]valine in 70% yield from the ester **32**. This approach may be extended to the synthesis of L-valine not only labelled in either diastereotopic methyl group, but also at C-2 and C-3 starting from the [1′,2′-$^{13}C_2$]-acylated chiral auxiliary, the synthesis of which has been described previously.[29]

Although this approach to the synthesis of L-α-amino acids with an asymmetric centre at C-3 is flexible and may be readily adapted for the incorporation of a range of isotopic labelling patterns, it did not lend itself to the introduction of a carbon-13 label at C-1 for which an excess of [^{13}C](cyanomethylene)-triphenylphosphorane would be required. Thus an alternative approach to such labelled compounds was required. Oppolzer and co-workers[30] have shown that (*E*)-α,β-unsaturated enoyl sultams will undergo conjugate addition with either organomagnesium or organocopper reagents. Trapping of the resultant enolate with 1-chloro-1-nitrosocyclohexane (CNC) provides a route to α-amino acids with an asymmetric centre at C-3 and indeed isoleucine was prepared in good yield with 90% de at C-3 and solely the *S*-configuration at C-2. This approach has been used extensively by Voges and co-workers to prepare a range of isotopically labelled amino acids.[31]

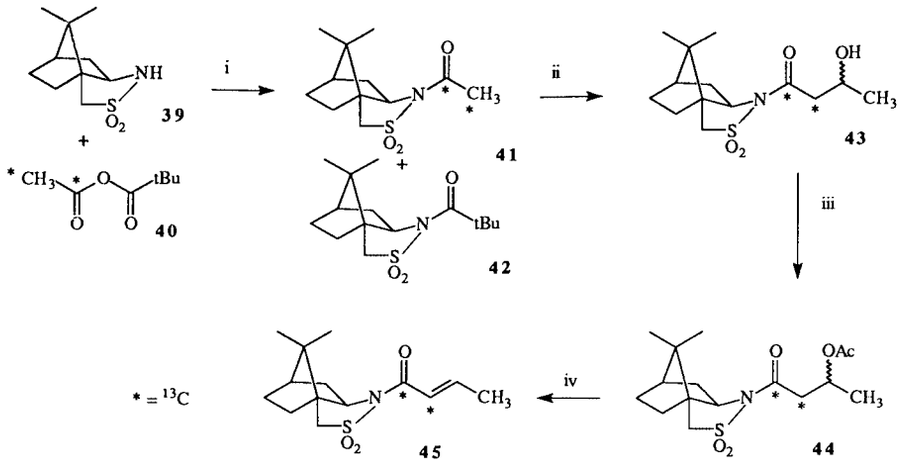
More recently we have shown that treatment of **34** with 2.6 equivalents of isotopically labelled Gilman reagent {prepared from [^{13}C]iodomethane, lithium metal and copper(I) iodide–tri-*n*-butylphosphine complex} at −78 °C gave the conjugate addition product **35** in 89% yield and *ca.* 82% de (Scheme 6).[29] From this intermediate, introduction of a nitrogen containing functionality at C-2 *via* an electrophilic amination reaction would generate a protected valine derivative. There are many electrophilic nitrogen equivalents available including diethyl azodicarboxylate (DEAD)[32] and di-*tert*-butyl azodicarboxylate (DBAD).[33] Amination of the sodium enolate of **35** with DBAD gave a 10:1 ratio of bornanesultam **36** and its diastereomer **37** in 79% yield which were separated by column chromatography. Cleavage of the auxiliary in **36** with lithium hydroperoxide gave acid **38** in 84% yield. The final step involved removal of the *tert*-butyloxycarbonyl groups using trifluoroacetic acid and hydrogenation of the hydrazine under medium pressure conditions using platinum oxide as a catalyst. After purification by ion exchange chromatography D-valine was isolated in 55% yield. The complementary diastereomer of valine can be synthesised starting from the (1*R*)-bornanesultam auxiliary. The formation of D-[^{15}N]valine can be achieved by using nitrogen-15 labelled di-*tert*-butyl azodicarboxylate.[34]

This approach can be adapted for the inclusion of carbon-13 at C-1 and C-2 of valine by formation of isotopically labelled crotonylbornanesultam as shown in Scheme 7. Acylation of sultam **39** using the mixed anhydride **40**, prepared from sodium [1,2-$^{13}C_2$]acetate and pivaloyl chloride, gave the acetylated bornanesultam **41** in 80% yield. An aldol reaction between the titanium enolate of **41** and acetaldehyde gave alcohol **43** in 94% yield as a 3:1 mixture of diastereomers. Acetylation of **43**, followed by elimination gave the desired isotopically labelled unsaturated sultam **45** in 86% yield over the two steps. This chemistry may be conducted efficiently on a multigram scale and used in the synthesis of [1,2-$^{13}C_2$]valine. Using [1,2-$^{13}C_2$]-acetaldehyde, this stepwise formation of the valine backbone enables the selective labelling at every position. This approach can be adapted for the synthesis of L-isoleucine and L-*allo*-isoleucine selectively labelled at each site.

Experimental

General experimental details have been described previously.[35] All NMR spectra were recorded in deuteriochloroform unless otherwise stated.

The enzymes were purchased and stored as follows: lipase from *Candida rugosa* (CRL), Sigma, stored at 4 °C as a 10000 eU mL^{-1} solution in tris buffer (5 mM); formate dehydrogenase (FDH) from *Candida boidinii*, Boerhinger, stored at 4 °C; leucine dehydrogenase (LeuDH) from *Bacillus* species, Sigma, stored at −20 °C; phenylalanine dehydrogenase (PheDH) from *Sporosarcina* species, Sigma, protein (50 eU) was dissolved in phosphate buffer (1 mL, 5 mM) and stored at −20 °C;



Scheme 7 *Reagents*: i, BuLi; ii, iPr_2EtN, $TiCl_4$, CH_3CHO; iii, Ac_2O, py; iv, DBU.

L-lactate dehydrogenase from *Bacillus stearothermophilus* (*BS*-LDH), Genzyme, stored at −20 °C; D-hydroxyisocaproate dehydrogenase from *Lactobacillus delbruekii* subsp. *bulgaricus* (*LB*-hicDH), gift from Professor J. J. Holbrook, Department of Biochemistry, University of Bristol, stored at −4 °C. β-Nicotinamide adenine dinucleotide hydride (NADH), Genzyme, stored at −20 °C.

General procedure for the one-pot hydrolysis and reductive amination of 2-keto esters

Potassium phosphate buffer (5 mM, 25 mL per mmol of substrate) was deoxygenated by bubbling through nitrogen for 0.5 h. CRL (10000 eU per mmol of substrate) and the keto ester (1 eq.) in ethanol (1 mL per mmol of substrate) were added and the reaction mixture stirred at rt, maintaining the pH between 7.0 and 8.5 by the periodic addition of sodium hydroxide (1.0 or 0.1 M). Once the pH had stopped changing (*ca.* 5 h, *ca.* 1 eq. of sodium hydroxide having been added), ammonium formate (10 eq.), 1 M dithiothreitol (DTT, 1 μL per mmol of substrate), formate dehydrogenase (8 mg, 4 eU for less or 16 mg, 8 eU for more than 3 mmol of substrate), NADH (8 mg, 12 μmol for less or 16 mg, 24 μmol for more than 3 mmol of substrate) and leucine dehydrogenase (5 eU for less or 10 eU for more than 3 mmol of substrate) were added. The resultant solution was stirred at rt and maintained between pH 7.0 and 7.5 by the periodic addition of hydrochloric acid (1.0 or 0.1 M) until the pH remained static (*ca.* 7 days, *ca.* 0.8 eq. of hydrochloric acid having been added). The reaction mixture was concentrated *in vacuo* and the product isolated by ion exchange chromatography on Dowex® 50WX8-100 (20 g per mmol of substrate), eluting first with water (3 × 40 mL per mmol of substrate) then conc. ammonia (2 × 100 mL per mmol of substrate). The conc. ammonia eluent was evaporated to dryness to yield the pure α-amino acid.

Cleavage of the auxiliary to prepare carboxylic acids (*S*)-4, (*R*)-4 and 30

Hydrogen peroxide (30%, 3 mL, 20 mmol) then lithium hydroxide (352 mg, 8 mmol) in water (10 mL) were added dropwise to (4*S*)-3-[(2*S*)-2-methylbutyryl]-4-isopropyloxazolidin-2-one **3** (1.06 g, 5.0 mmol) in THF (20 mL) and water (10 mL) at 0 °C. The reaction was stirred at 0 °C for 2 h then sodium sulfite (2.5 g) in water (10 mL) was added. The reaction mixture was stirred at 0 °C for 0.25 h then acidified to pH 1 with sulfuric acid (2 M). The products were extracted with ether, dried over magnesium sulfate, filtered and concentrated i*n vacuo* to *ca.* 5 mL then separated by high vacuum distillation giving the chiral auxiliary (*S*)-4-isopropyloxazolidin-2-one **1** as the residue and (*S*)-2-methylbutanoic acid (*S*)-**4** (466 mg, 91%) as the distillate as a colourless oil; $[a]_D^{22}$ +17 (neat) [lit.,[35] +19 (neat)]; ν_{max} (Nujol)/cm^{-1} 3443 (O–H), 2973 (C–H), 1709 (C=O); δ_H (270 MHz) 9.5 (1H, br s, COOH), 2.40 (1H, sextet, *J* 7.0, 2-H), 1.71 (1H, m, 3-*H*H), 1.50 (1H, m, 3-H*H*), 1.18 (3H, d, *J* 7.0, 2-CH_3), 0.95 (3H, t, *J* 7.4, 4-H_3); *m*/*z* 101 (M^+ − 1, 1%), 86 (30), 74 (48) and 58 (100).

The above reaction was carried out using (4*R*,5*S*)-4-methyl-3-[(*R*)-2-methylbutyryl]-5-phenyloxazolidin-2-one **10** (781 mg, 3.0 mmol). Separation and purification by high vacuum distillation gave the auxiliary (4*R*,5*S*)-4-methyl-5-phenyloxazolidin-2-one **8** as a white solid (540 mg, 100%) and (*R*)-2-methylbutanoic acid (*R*)-**4** as a colourless oil (300 mg, 98%); $[a]_D^{25}$ −14 (neat), [lit.,[36] −18 (neat)]; spectral data as above.

The above reaction was carried out using (4*S*)-4-isopropyl-3-{(2*R*)-2-methyl[3,3,3-D_3]propionyl}oxazolidin-2-one **29** (2.02 g, 10 mmol) giving, after purification by column chromatography, the separated auxiliary (*S*)-4-isopropyloxazolidin-2-one **1** (1.163 g, 90%) and (*R*)-2-[3,3,3-D_3]methylpropanoic acid **30** (715 mg, 78%) as a colourless oil; $[a]_D^{24}$ +1.0 (*c* 4.9 in $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 3436 (C–O), 2924 (C–H) and 1627 (C=O); δ_H (270 MHz) 11.2 (1H, br s, COOH), 2.52 (1H, br q, *J* 6.8, 2-H), 1.22 (3H, d, *J* 6.8, CH_3); δ_C (67.5 MHz) 184.0 (C-1), 33.7 (C-2), 18.6 (CH_3), 17.8 (quintet, *J* 19.2, CD_3); *m*/*z* 91 (M^+, 17%), 83 (100), 69 (9) and 57 (15); 95% incorporation of three deuterium atoms by mass spectrometry.

Preparation of β-ketocyanophosphoranes (*S*)-5, (*R*)-5, 19 and 31

(Cyanomethylene)triphenylphosphorane hydrochloride (9.45 g, 28 mmol), prepared as previously reported,[37] was dissolved in water (60 mL) and the solution washed with dichloromethane (60 mL). The aqueous layer was stirred with sodium hydroxide [1.68 g, 42 mmol in water (30 mL)] and dichloromethane (60 mL) for 10 min at rt. The layers were separated and the aqueous layer was extracted with dichloromethane (60 mL). The combined organic extracts were dried over magnesium sulfate, filtered and evaporated until a precipitate just formed. Sufficient dichloromethane was added to redissolve the precipitate.

The above ylide in dichloromethane was added to 4-dimethylaminopyridine (DMAP) (110 mg, 0.9 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (2.7 g, 14 mmol) and stirred together under nitrogen. (*S*)-2-Methylbutanoic acid (*S*)-**4** (1.02 g, 10.0 mmol) in dichloromethane (10 mL) was added dropwise and the reaction stirred at room temperature for 16 h. Water was added and the products extracted into dichloromethane. The organic layer was washed with saturated aqueous sodium hydrogen carbonate, brine, dried over magnesium sulfate and concentrated *in vacuo* to give a mixture of the β-ketocyanophosphorane (*S*)-**5** (8.31 g) and triphenylphosphine oxide. This was used without purification for the

ozonolysis, although a pure sample of *(S)-4-methyl-3-oxo-2-triphenylphosphoranylidenehexanenitrile* (*S*)-**5** could be obtained by column chromatography; mp 183–184 °C; $[a]_D$ +23.6 (*c* 4.0 in $CHCl_3$) (Found: C, 77.9; H, 6.5; N, 3.6; P, 7.8. $C_{25}H_{24}NOP$ requires C, 77.9; H, 6.2; N, 3.6; P, 8.05%); ν_{max} ($CHCl_3$)/cm^{-1} 2168 (CN), 1579 (C=O), 1569 (C=C); δ_H (270 MHz) 7.7–7.4 (15H, m, aromatic), 3.12 (1H, m, 4-H), 1.71 (1H, m, 5-*H*H), 1.38 (1H, m, 5-H*H*), 1.13 (3H, d, *J* 6.9, 4-CH_3), 0.92 (3H, t, *J* 7.4, 6-H_3); *m*/*z* M$^+$ 385.1607 (M$^+$, 6%. $C_{25}H_{24}NOP$ requires 385.1596), 357 (1), 328 (100), 303 (8), 277 (8) and 183 (16).

The reaction was carried out using (*R*)-2-methylbutanoic acid (*R*)-**4** (408 mg, 4.0 mmol) and a sample was purified by column chromatography giving *(R)-4-methyl-3-oxo-2-triphenylphosphoranylidenehexanenitrile* as a white solid; mp 193–195 °C; $[a]_D^{24}$ −22.2 (*c* 3.0 in $CHCl_3$) (Found: C, 78.0; H, 6.1; N, 3.8; P, 8.1. $C_{25}H_{24}NOP$ requires C, 77.9; H, 6.2; N, 3.6; P, 8.0%). All spectroscopic data are the same as above.

The reaction was carried out using the known[37] (*S*)-2-benzyloxypropanoic acid **18** (14.4 g, 80 mmol). Purification by flash column chromatography, eluting with 40% ethyl acetate in light petroleum gave a light brown solid. Recrystallisation of the brown solid from petroleum ether and dichloromethane (1:1) gave *(S)-4-benzyloxy-3-oxo-2-triphenylphosphoranylidenepentanenitrile* **19** (22.5 g, 73%) as pale brown crystals; mp 213–215 °C (from dichloromethane and light petroleum); $[a]_D^{25}$ −33 (*c* 2.1 in $CHCl_3$) (Found: C, 77.6; H, 5.7; N, 2.9. $C_{30}H_{26}NO_2P$ requires C, 77.8; H, 5.6, N, 3.0%); ν_{max}(Nujol)/cm^{-1} 2176 (CN), 1594 (C=O); δ_H (270 MHz) 1.52 (3H, d, *J* 7.0, 5-H_3), 4.44 (1H, d, *J* 11.0, PhC*H*H), 4.64 (1H, q, *J* 7.0, 4-H), 4.69 (1H, d, *J* 11.0, PhCH*H*) and 7.20–7.65 (20H, m, aromatics); *m*/*z* (CI) 464 ([MH]$^+$, 100%), 386 (9), 357 (30), 344 (8) and 328 (51).

The reaction was carried out using (*R*)-2-methyl[3,3,3-D_3]-propanoic acid **30** (546 mg, 6 mmol), affording a mixture of triphenylphosphine oxide and β-ketocyanophosphorane **31** as a white solid (5.54 g). A pure sample of *(R)-4-methyl-3-oxo-2-triphenylphosphoranylidene[5,5,5-D_3]pentanenitrile* **31** was obtained after column chromatography; mp 195–197 °C (from dichloromethane and light petroleum) (Found: C, 77.1; H, 6.8; N, 3.7; P, 8.0. $C_{24}H_{19}D_3NOP$ requires C, 77.0; H, 6.7; N, 3.7; P, 8.3%); $[a]_D^{24}$ +0.5 (*c* 5.0 in $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 2175 (CN), 1578 (C=O) and 1563 (C=C); δ_H (270 MHz) 7.70–7.45 (15H, m, aromatic), 3.29 (1H, br q, *J* 6.6, 4-H), 1.14 (3H, d, *J* 6.6, CH_3); *m*/*z* 374.1621 (M$^+$, 6%. $C_{24}H_{19}D_3NOP$ requires 374.1628), 328 (100), 277 (9), 201 (6), 183 (17) and 165 (6); 94% incorporation of 3 isotopic labels and 6% incorporation of 2 isotopic labels by mass spectrometry.

Preparation of 2-keto esters (*S*)-6, (*R*)-6, 20 and 32

Crude (*S*)-4-methyl-3-oxo-2-triphenylphosphoranylidenehexanenitrile (*S*)-**5** (8 g) in methanol (30 mL) and dichloromethane (70 mL) was cooled to −78 °C. Ozone was bubbled through the reaction mixture until the solution turned blue. Oxygen was bubbled through the solution for 5 minutes, then the reaction mixture was warmed to room temperature. The reaction mixture was concentrated *in vacuo* until a solid started to precipitate. Purification by high vacuum distillation gave, after removal of the solvent, *methyl (S)-3-methyl-2-oxopentanoate* (*S*)-**6** (468 mg, 75% yield from (*S*)-**4**) as a colourless oil; $[a]_D$ +25.5 (*c* 4.0 in $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1732 (br); δ_H (500 MHz) 3.88 (3H, s, OCH_3), 3.16 (1H, sextet, *J* 6.9, 3-H), 1.77 (1H, m, 4-*H*H), 1.45 (1H, m, 4-H*H*), 1.14 (3H, d, *J* 6.9, 3-CH_3), 0.92 (3H, t, *J* 7.5, 5-H_3); δ_C (125 MHz) 197.7 (C-2), 162.1 (C-1), 52.6 (O-CH_3), 43.4 (C-3), 24.7 (C-4), 14.4 (3-CH_3), 11.2 (C-5); *m*/*z* 144 (M$^+$, 3%), 113 (31), 97 (25), 85 (58), 71 (57) and 57 (100).

The above reaction was carried out using unpurified (*R*)-4-methyl-3-oxo-2-triphenylphosphoranylidenehexanenitrile, affording after purification by high vacuum distillation, *methyl (R)-3-methyl-2-oxopentanoate* (*R*)-**6** (355 mg, 62% yield from (*R*)-**4**) as a colourless oil; $[a]_D$ −20.5 (*c* 4.9 in $CHCl_3$); spectroscopic data as above.

The above reaction was carried out using (*S*)-4-benzyloxy-3-oxo-2-triphenylphosphoranylidenepentanenitrile **19** (0.21 g, 0.45 mmol). Purification by flash column chromatography, eluting with 25% ethyl acetate in light petroleum gave *methyl (S)-3-benzyloxy-2-oxobutanoate* **20** (0.076 g, 75%) as a viscous colourless oil; $[a]_D^{24}$ −6.9 (*c* 1.0 in $CHCl_3$); ν_{max} (film)/cm^{-1} 3068 (CH), 2655, 1742 (br, 2 × C=O); δ_H (270 MHz) 1.44 (3H, d, *J* 7.0, 4-H_3), 3.84 (3H, s, OCH_3), 4.55 (1H, q, *J* 7.0, 3-H), 4.55 (1H, d, *J* 11.0, PhC*H*H), 4.66 (1H, d, *J* 11.0, PhCH*H*) and 7.39 (5H, m, aromatics); δ_C (75.5 MHz) 16.5 (C-4), 52.8 (OMe), 72.6 (C-3), 76.6 (Ph*C*H_2), 128.0, 128.1, 128.5, 137.1 (aromatics), 162.7 (C-1), 195.0 (C-2); *m*/*z* (CI) 223 ([MH]$^+$, 0.3%), 221 (1.5), 181 (30), 119 (6), 105 (7) and 91 (100).

The above reaction was carried out using unpurified (*R*)-4-methyl-3-oxo-2-triphenylphosphoranylidene-[5,5,5-D_3]-pentanenitrile **31** (5.54 g). This gave, after purification by high vacuum distillation, *methyl (R)-3-methyl-2-oxo[4,4,4-D_3]-butanoate* **32** (504 mg, 63% yield from **30**) as a colourless liquid; $[a]_D^{23}$ +1.5 (*c* 2.0 in $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1738 (C=O); δ_H (270 MHz) 3.88 (3H, s, OCH_3), 3.26 (1H, br q, *J* 7.0, 3-H), 1.16 (3H, d, *J* 7.0, 3-CH_3); δ_C (67.5 MHz) 197.6 (C-2), 161.8 (C-1), 52.4 (OCH_3), 36.5 (C-3), 16.7 (3-CH_3), 16.0 (quintet, *J* 19.6, CD_3); *m*/*z* 133 (M$^+$, 12%), 97 (28), 83 (34), 71 (66) and 57 (100); 95% incorporation of 3 deuterium atoms by mass spectrometry.

Methyl (*S*)-3-methoxy-2-oxobutanoate 14

The known acid (*S*)-2-methoxypropanoic acid **12** (0.5 g, 5 mmol) was converted to (*S*)-4-methoxy-3-oxo-2-(triphenylphosphoranylidene)pentanenitrile **13** *via* coupling with (cyanomethylene)triphenylphosphorane using the procedure described above. The crude product as a mixture with triphenylphosphine oxide was then ozonolysed as described above. Purification by flash column chromatography, eluting with 43% ethyl acetate in light petroleum gave *2-keto ester* **14** (0.15 g, 20% over two steps) as a colourless oil; $[a]_D^{25}$ −7.7 (*c* 2.0 in $CHCl_3$); ν_{max} (film)/cm^{-1} 2985 (CH), 2832, 1736 (br, 2 × C=O); δ_H (270 MHz) 1.42 (3H, d, *J* 7.0, 4-H_3), 3.42 (3H, s, OCH_3), 3.90 (3H, s, CO_2CH_3) and 4.39 (1H, q, *J* 7.0, 3-H); *m*/*z* (CI) 147.0664 (MH$^+$, 10%. $C_6H_{11}O_4$ requires 147.0665, 10%), 129 (5), 115 (11), 95 (20), 87 (58) and 84 (62).

L-[^{15}N]Isoleucine

The biotransformations were carried out according to the general procedure using methyl (*S*)-3-methyl-2-oxopentanoate (*S*)-**6** (147 mg, 1.02 mmol) and [^{15}N]ammonium formate giving, after purification by ion exchange chromatography, L-[^{15}N]isoleucine (100 mg, 74%) as a white powder; $[a]_D^{24}$ +36.3 (*c* 5.0 in 6 M HCl) [lit. of unlabelled material,[38] +40.7 (*c* 4.0 in 6 M HCl)]; δ_H (500 MHz; D_2O) 4.00 (1H, d, *J* 3.8, 2-H), 2.03 (1H, m, 3-H), 1.46 and 1.30 (2 × 1H, each m, 4-H_2), 0.99 (3H, d, *J* 7.0, 3-CH_3), 0.91 (3H, t, *J* 7.4, 5-H_3); δ_C (125 MHz; D_2O) 173.1 (C-1), 58.6 (d, *J* 4.1, C-2), 35.5 (C-3), 24.2 (C-4), 14.2 (3-CH_3), 10.8 (C-5); *m*/*z* 132 (M$^+$, 1%), 103 (10), 87 (100), 76 (67) and 58 (55); 96% incorporation of nitrogen-15 by mass spectrometry.

L-*allo*-[^{15}N]Isoleucine

The biotransformations were carried out according to the general procedure using methyl (*R*)-3-methyl-2-oxopentanoate (*R*)-**6** (147 mg, 1.02 mmol) and [^{15}N]ammonium formate affording, after purification by ion-exchange chromatography, L-*allo*-[^{15}N]isoleucine (100 mg, 74%) as a white powder; $[a]_D^{22}$ +39.1 (*c* 1.0 in 6 M HCl) [lit.,[39] +40.5 (*c* 1.0 in 5 M HCl), unlabelled

material]; δ_H (500 MHz; D_2O) 3.94 (1H, d, *J* 3.8, 2-H), 2.06 (1H, m, 3-H), 1.40 (1H, m, 4-H*H*), 1.27 (1H, m, 4-*H*H), 0.90 (3H, d, *J* 7.1, 3-CH_3), 0.88 (3H, t, *J* 7.4, 5-H_3); *m*/*z* 132 (M^+, 1%), 115 (2), 103 (1), 87 (100), 76 (42) and 58 (41); 97% incorporation of nitrogen-15 by mass spectrometry.

(2*S*,3*S*)-2-Amino-3-methoxybutanoic acid 16

The biotransformations were carried out according to the general procedure using methyl (*S*)-3-methoxy-2-oxobutanoate **14** (0.15 g, 1.0 mmol). Purification by ion exchange chromatography gave (2*S*,3*S*)-2-amino-3-methoxybutanoic acid **16** (0.10 g, 73%) as a pale brown solid; $[\alpha]_D^{24}$ +29.0 (*c* 1.7 in 6 M HCl) [lit.,[40] $[\alpha]_D^{23}$ +30.5 (*c* 2.0 in 5 M HCl)]; δ_H (270 MHz; D_2O) 1.20 (3H, d, *J* 7, 4-H_3), 3.40 (3H, s, OCH_3), 3.96 (1H, qd, *J* 7, 4, 3-H), 4.12 (1H, d, *J* 4, 2-H); *m*/*z* (CI) 134 (MH^+, 6%), 132 (10), 117 (8) and 104 (100).

Methyl (2*S*,3*S*)-3-benzyloxy-2-hydroxybutanoate 24

Methyl (*S*)-3-benzyloxy-2-oxobutanoate **20** (0.222 g, 1.0 mmol) was dissolved in 5 mM tris buffer (40 mL). *Candida rugosa* lipase (10000 eU) was added and the pH adjusted to 7.5 by the addition of 1.0 M hydrochloric acid. The pH was maintained at a value between 7.0 and 7.5 by the addition of 0.1 M sodium hydroxide until the pH stopped changing. The solution was then deoxygenated by bubbling through a stream of nitrogen for 1 h. DTT (20 μL) was added followed by *BS*-LDH (10 mg), FDH (10 mg), sodium formate (1 g) and NADH (10 mg). The reaction was left stirring under a nitrogen atmosphere with the pH being kept constant at about 6.1 by the addition of 1.0 M hydrochloric acid. After the pH stopped changing the reaction mixture was acidified to pH 2 by the addition of 2 M hydrochloric acid and extracted with ethyl acetate (3 × 40 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting 2-hydroxy acid was methylated with ethereal diazomethane and purification by flash column chromatography, eluting with 35% ethyl acetate in light petroleum gave *methyl (2S,3S)-3-benzyloxy-2-hydroxybutanoate* **24** (0.216 g, 96%) as a viscous oil; $[\alpha]_D^{24}$ +18.5 (*c* 0.5 in $CHCl_3$); ν_{max} (film)/cm^{-1} 3427 (OH), 2979 (CH), 1736 (C=O); δ_H (270 MHz) 1.21 (3H, d, *J* 6.0, 4-H_3), 2.92 (1H, d, *J* 6.0, OH), 3.79 (3H, s, OCH_3), 3.86 (1H, qd, *J* 6.0, 3.0, 3-H), 4.34 (1H, dd, *J* 6.0, 3.0, 2-H), 4.57 (1H, d, *J* 12.0, PhC*H*H), 4.63 (1H, d, *J* 12.0, PhCH*H*), 7.26–7.38 (5H, m, Ph); *m*/*z* (CI) 225.1111 (MH^+, 2%. $C_{12}H_{16}O_4$ requires 225.1127), 209 (2), 195 (3), 181 (3), 119 (10), 105 (7) and 91 (100).

Methyl (2*R*,3*S*)-3-benzyloxy-2-hydroxybutanoate 25

The above reaction was repeated using methyl (*S*)-3-benzyloxy-2-oxobutanoate **20** (0.222 g, 1.0 mmol) and *LB*-hicDH in place of *BS*-LDH. Purification by flash column chromatography, eluting with 35% ethyl acetate in light petroleum gave *methyl (2R,3S)-3-benzyloxy-2-hydroxybutanoate* **25** (0.213 g, 95%) as a viscous oil; $[\alpha]_D^{26}$ +13.6 (*c* 1.2 in $CHCl_3$); ν_{max} (film)/cm^{-1} 3464 (OH), 2980 (CH), 1741 (C=O); δ_H (300 MHz) 1.36 (3H, d, *J* 6.0, 4-H_3), 2.95 (1H, d, *J* 6.0, OH), 3.71 (3H, s, OCH_3), 3.92 (1H, qd, *J* 6.0, 3.0, 3-H), 4.07 (1H, dd, *J* 6.0, 3.0, 2-H), 4.39 (1H, d, *J* 12.0, PhC*H*H), 4.61 (1H, d, *J* 12.0, PhCH*H*), 7.25–7.36 (5H, m, Ph); *m*/*z* (CI) 225.1121 (MH^+, 3%. $C_{12}H_{16}O_4$ requires 225.1127), 219 (7), 195 (8), 181 (12), 135 (7), 101 (21) and 91 (100).

L-*allo*-Threonine

The biotransformations were carried out according to the general procedure using methyl (*S*)-3-methoxymethoxy-2-oxobutanoate (*S*)-**26** (0.176 g, 1.0 mmol). Purification by ion exchange chromatography gave L-*allo*-threonine (0.11 g, 93%) as a pale yellow solid; $[\alpha]_D^{22}$ +8.6 (*c* 1.0 in H_2O) [lit.,[41] $[\alpha]_D^{24}$ +9.3 (*c* 3.8 in H_2O)]; δ_H(400 MHz; D_2O) 1.19 (3H, d, *J* 7.0, 4-H_3), 3.84 (1H, d, *J* 4.0, 2-H), 4.34 (1H, qd, *J* 7.0, 4.0, 3-H); *m*/*z* (CI) 120 (MH^+, 3%), 107 (6), 105 (65), 85 (23) and 77 (52).

L-*allo*-[^{15}N]Threonine

The biotransformations were carried out according to general procedure using methyl (*S*)-3-methoxymethoxy-2-oxobutanoate (*S*)-**26** (0.176 g, 1.0 mmol) and [^{15}N]ammonium formate (0.08 g, 1.2 mmol). Purification by ion exchange chromatography gave L-*allo*-[^{15}N]threonine (0.11 g, 93%) as a pale yellow solid. Spectroscopic data were as above except for mass spectrometry; *m*/*z* (CI) 121 (MH^+, 15%), 104 (75), 85 (25) and 76 (100); 98% incorporation of ^{15}N by mass spectrometry.

L-Threonine

The biotransformations were carried out according to general procedure using methyl (*R*)-3-methoxymethoxy-2-oxobutanoate (*R*)-**26** (0.176 g, 1.0 mmol) and phenylalanine dehydrogenase. Purification by ion exchange chromatography gave L-threonine (0.07 g, 61%) as a pale yellow solid; $[\alpha]_D^{25}$ −27.2 (*c* 0.1 in H_2O) [lit.,[41] $[\alpha]_D^{27}$ −28.3 (*c* 6.0, H_2O)]; δ_H (270 MHz; D_2O) 1.27 (3H, d, *J* 7.0, 4-H_3), 3.54 (1H, d, *J* 4.0, 2-H), 4.21 (1H, qd, *J* 7.0, 4.0, 3-H); *m*/*z* (CI) 120 (MH^+, 5%), 104 (75), 85 (31) and 76 (100).

L-[^{15}N]Threonine

The biotransformations were carried out according to the general procedure using methyl (*R*)-3-methoxymethoxy-2-oxobutanoate (*R*)-**26** (0.176 g, 1.0 mmol), [^{15}N]ammonium formate (0.08 g, 1.2 mmol) and phenylalanine dehydrogenase. Purification by ion exchange chromatography gave L-[^{15}N]-threonine (0.07 g, 61%) as a pale yellow solid. Spectroscopic data were as above except for mass spectrometry; *m*/*z* (CI) 121 (MH^+, 17%), 104 (62), 86 (29) and 76 (100); 98% incorporation of ^{15}N by mass spectrometry.

(2*S*,3*R*)-[4,4,4-D_3]Valine

The biotransformations were carried out according to the general procedure using methyl (*R*)-[4,4,4-D_3]-3-methyl-2-oxobutanoate **32** (133 mg, 1.0 mmol) giving, after purification by ion exchange chromatography, (2*S*,3*R*)-[4,4,4-D_3]valine (96 mg, 80%) as a white powder; $[\alpha]_D^{23}$ +23.0 (*c* 1.5 in 6 M HCl) [lit.,[28] (2*R*,3*S*)-enantiomer −25.0 (*c* 0.6 in aq. HCl)]; δ_H (500 MHz; D_2O) 3.87 (1H, d, *J* 4.4, 2-H), 2.25 (1H, m, 3-H), 0.94 (3H, d, *J* 7.0, 3-CH_3); δ_C (75 MHz; D_2O + d_6-acetone) 172.3 (C-1), 59.1 (C-2), 29.7 (C-3), 17.7 (3-CH_3), 17.3 (quintet, *J* 20.4, CD_3); *m*/*z* 102 (M^+ − 18, 1%), 86 (1), 75 (100) and 57 (29); 93% incorporation of three deuterium atoms by mass spectrometry.

(2*S*,3*R*)-[^{15}N-4,4,4-D_3]Valine

The biotransformations were carried out according to the general procedure using methyl (*R*)-[4,4,4-D_3]-3-methyl-2-oxobutanoate **32** (135 mg, 1.01 mmol) and [^{15}N]ammonium formate giving, after purification by ion exchange chromatography (2*S*,3*R*)-[^{15}N-4,4,4-D_3]valine (86 mg, 70%) as a white powder; $[\alpha]_D^{25}$ +24.6 (*c* 1.5 in 6 M HCl) [lit.,[39] +28.5 (*c* 1 in 5 M HCl) for L-[^{15}N]valine]; δ_H (500 MHz; D_2O) 3.89 (1H, d, *J* 4.4, 2-H), 2.27 (1H, m, 3-H), 0.95 (3H, d, *J* 7.0, 3-CH_3); δ_C (75 MHz; D_2O + d_6-acetone) 172.1 (C-1), 59.1 (d, *J* 5.6, C-2), 29.6 (C-3), 17.7 (3-CH_3), 17.3 (quintet, *J* 19.8, CD_3); *m*/*z* 103 (M^+ − 17, 1%), 86 (1), 76 (100) and 58 (30); 94% incorporation of four isotopic labels by mass spectrometry.

(1*S*,2*R*)-*N*-{(*R*)-2′-[1″,2″-Bis(butoxycarbonyl)hydrazino]-3′-methylbutanoyl}bornane-10,2-sultam 36

A 1 M solution of sodium hexamethyldisilazide in THF (1.47 mL, 1.47 mmol) was added to a stirred solution of the known[29] (1*S*,2*R*)-*N*-(3′-methylbutanoyl)bornane-10,2-sultam **35** (402

mg, 1.34 mmol) in dry THF (10 mL) at −78 °C under nitrogen in a flame dried flask. The solution was stirred at −78 °C for 1 h. Di-*tert*-butyl azodicarboxylate (DBAD) (0.451 g, 1.67 mmol) in dry dichloromethane (15 mL) was added slowly (over 20 minutes) at −78 °C to the enolate solution. The reaction mixture was stirred for a further 2 h at −78 °C, before being quenched with glacial acetic acid (10 mL). After warming to room temperature, the reaction mixture was partitioned between water (60 mL) and dichloromethane (60 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic phase was washed with saturated aqueous sodium hydrogen carbonate (60 mL), dried (Na_2SO_4) and the solvents were removed *in vacuo*. Purification by column chromatography eluting with 0–30% ethyl acetate–petrol (40–60) gave two main fractions. The less polar major fraction isolated was *(1S,2R)-N-{(R)-2′-[1″,2″-bis(butoxycarbonyl)-hydrazino]-3′-methylbutanoyl}bornane-10,2-sultam* **36** (0.658 g, 71%) as a white solid; mp 74–76 °C; $[\alpha]_D^{24}$ −2.54 (*c* 1.37 in $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 3355 (NH), 1755 (C=O), 1706 (C=O), 1343 (SO_2), 1149 (SO_2); δ_H (270 MHz, 55 °C) 0.96 and 1.20 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.00 (6H, m, 3′-CH_3 and 4′-H_3), 1.44 and 1.46 (2 × 9H, 2 × s, 2 × -$C(CH_3)_3$), 1–2.2 (7H, m, 3-H_2, 4-H, 5-H_2 and 6-H_2), 2.33 (1H, m, 3′-H), 3.38 (1H, d, *J* 14.0, 10-*H*H), 3.46 (1H, d, *J* 14.0, 10-H*H*), 4.00 (1H, br s, 2-H), 4.81 (1H, br s, 2′-H), 6.28 (1H, br s, N-H); δ_C (75 MHz) 18.6, 19.1, 20.0 and 21.0 (C-8, C-9, 3′-CH_3 and C-4′), 26.5 (C-3, C-5 or C-6), 26.6 (C-3′), 28.0 and 28.2 (each C(*C*H_3)$_3$), 32.8 and 38.6 (C-3, C-5 or C-6), 44.8 (C-4), 47.7 and 48.2 (C-1 and C-7), 53.2 (C-10), 65.5 (C-2), 80.8 and 82.0 (each *C*$(CH_3)_3$), 116.0 (C-2′), 154.4, 164.3 and 168.0 (C-1′and each C=O); *m/z* (CI) 530.2899 (MH^+, 6%. $C_{25}H_{44}N_3O_7S$ requires 530.2900), 464 (6), 418 (58), 374 (34), 330 (84) and 216 (100).

The more polar minor fraction isolated was *(1S,2R)-N-{(S)-2′-[1″,2″-bis(butoxycarbonyl)hydrazino]-3′-methylbutanoyl}-bornane-10,2-sultam* **37** (49 mg, 6.9%) as a pale yellow gum which solidified to a white solid over time; $[\alpha]_D^{24}$ −38.8 (*c* 0.91 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3351 (NH), 1715 (C=O), 1697 (=CO), 1333 (SO_2), 1147 (SO_2); δ_H (270 MHz; 55 °C) 0.96 and 1.20 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.03 and 1.05 (2 × 3H, 2 × d, *J* 7.0, 3′-CH_3 and 4′-H_3), 1.20–1.60 (2H, m), 1.46 and 1.47 (2 × 9H, 2 × s, 2 × -$C(CH_3)_3$), 1.89 (3H, m), 2.08 (2H, m), 2.40 (1H, m, 3′-H), 3.40 (1H, d, *J* 14.0, 10-*H*H), 3.47 (1H, d, *J* 14.0, 10-H*H*), 3.90 (1H, t, *J* 6.0, 2-H), 5.11 (1H, d, *J* 8.4, 2′-H), 6.48 (1H, br s, N-H); δ_C (75 MHz) 18.7 (3′-CH_3 and C-4′), 19.5 and 20.1 (C-8 and C-9), 26.1, 32.3 and 37.8 (C-3, C-5 and C-6), 27.6 and 27.8 (each C(*C*H_3)$_3$), 29.6 (C-3′), 44.1 (C-4), 47.4 and 48.0 (C-1 and C-7), 52.6 (C-10), 64.7 (C-2), 77.2 (C-2′), 80.7 and 81.3 (each O*C*$(CH_3)_3$), 154.1 and 154.3 (each *C*OO^{t}Bu), 170.8 (C-1′); *m/z* (CI) 530.2916 (MH^+, 9%. $C_{25}H_{44}N_3O_7S$ requires 530.2900), 464 (5), 418 (100), 374 (86), 330 (98) and 216 (56).

(*R*)-2-[1′,2′-Bis(butoxycarbonyl)hydrazino]-3-methylbutanoic acid 38

Hydrogen peroxide solution (30%, 0.13 mL, 1.2 mmol) and lithium hydroxide monohydrate (32 mg, 0.76 mmol) were added successively to a solution of (1*S*,2*R*)-*N*-{(*R*)-2′-[1″,2″-bis(butoxycarbonyl)hydrazino]-3′-methylbutanoyl}bornane-10,2-sultam **36** (0.205 g, 0.39 mmol) in THF (2 mL) and water (1 mL) at rt. The mixture was stirred for 4 h at rt. The reaction mixture was cooled to 0 °C, before a solution of sodium sulfite (0.15 g) in water (3 mL) was added and the solution stirred at 0 °C for a further 15 minutes. The solution was adjusted to pH 9–10 with saturated aqueous sodium hydrogen carbonate, the THF was removed *in vacuo* and the residual aqueous solution was extracted with dichloromethane (3 × 20 mL). The aqueous solution was adjusted to pH 1–2 with hydrochloric acid (6 M) and extracted with ethyl acetate (3 × 40 mL). The combined extracts were dried (Na_2SO_4) and the solvents removed *in vacuo* to yield *(R)-2-[1′,2′-bis(butoxycarbonyl)hydrazino]-3-methylbutanoic acid* **38** (0.108 g, 84%) as a yellow gum; $[\alpha]_D$ +16.5 (*c* 2.18 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3389 (NH), 3019 and 2980 (CH), 1714 (C=O); δ_H (300 MHz) 1.03 (3H, m, 4-H_3), 1.09 (3H, d, *J* 6.8, 3-CH_3), 1.47 and 1.51 (2 × 9H, 2 × s, 2 × -$C(CH_3)_3$), 2.32 (1H, br s, 3-H), 3.83 (1H, br s, 2-H); *m/z* (CI) 333.2031 (MH^+, 1.5%. $C_{15}H_{29}N_2O_6$ requires 333.2056), 277 (18), 221 (45), 133 (88) and 57 (100).

D-Valine

(*R*)-2-[1′,2′-Bis(butoxycarbonyl)hydrazino]-3-methylbutanoic acid **38** (0.39 g, 1.2 mmol) was dissolved in dichloromethane (8 mL) and trifluoroacetic acid (3 mL). The mixture was stirred for 1 h, before the solvent was removed *in vacuo*. The trifluoroacetic acid was azeotroped off with toluene, ethyl acetate and chloroform to give a white solid. The residue was dissolved in distilled water (30 mL) and platinum dioxide (50 mg) was added. The reaction mixture was shaken under a hydrogen atmosphere at 35 psi overnight. The reaction mixture was filtered through Celite, which was washed with methanol (40 mL). The filtrate was concentrated and the resulting residue was purified using a Dowex 50W ion exchange column. The resulting ammonia washing was concentrated to give D-valine (76 mg, 55%) as a white solid; mp 295–298 °C (subl.) [lit.[42] 295 (subl.)]; $[\alpha]_D^{23}$ −28.8 (*c* 0.71 in 6 M HCl) [lit.,[43] −28.4 (*c* 2 in 6 M HCl)]; δ_H (270 MHz; D_2O) 0.89 and 0.94 (2 × 3H, 2 × d, *J* 7.0, 3-CH_3 and 4-H_3), 2.27 (1H, m, 3-H), 3.52 (1H, d, *J* 4.0, 2-H); δ_C (75 MHz; D_2O) 19.5 and 20.0 (3-CH_3 and C-4), 31.7 (C-3), 61.0 (C-2), 174.1 (C-1); *m/z* (CI) 118 (MH^+, 15%), 100 (11), 86 (9), 72 (100) and 57 (17).

(1*S*,2*R*)-*N*-(Acetyl)bornane-10,2-sultam 41

Trimethylacetyl chloride (0.2 mL, 1.65 mmol) was added to sodium acetate (0.123 g, 1.5 mmol, dried in the vacuum oven overnight at 100 °C) in THF (5 mL) in an oven dried flask under nitrogen. The reaction mixture was stirred at rt overnight, before cooling to −78 °C. Butyllithium (2.5 M solution in hexane, 0.8 mL, 1.8 mmol) was added slowly to (1*S*)-bornane-10,2-sultam **39** (0.355 g, 1.65 mmol) in THF (6 mL) at −78 °C in an oven dried flask under nitrogen, before stirring at −78 °C for 0.5 h. This reaction mixture was added slowly to the mixed anhydride **40** at −78 °C, before stirring for 3 h. Saturated aqueous ammonium chloride (20 mL) was added and the reaction mixture was allowed to warm to rt. The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (40 mL), dried ($MgSO_4$) and the solvents removed *in vacuo*. Purification by column chromatography eluting with 0–50% ethyl acetate–petrol (40–60) gave three main fractions. The least polar fraction gave **42** (0.104 g, 21.1% based on **39**) as a white solid; mp 133–134 °C (from MeOH); $[\alpha]_D$ −55.5 (*c* 1.18 in $CHCl_3$) (Found: C, 60.24; H, 8.61; N, 4.62; S, 10.79. $C_{15}H_{25}NO_3S$ requires C, 60.20; H, 8.36; N, 4.68; S, 10.70%); ν_{max} (CH_2Cl_2)/cm^{-1} 1688 (C=O); δ_H (300 MHz) 0.95 and 1.15 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.34 [9H, s, C$(CH_3)_3$], 1.35 (2H, m), 1.89 (5H, m, 3-H_2, 4-H, 5-H_2 and 6-H_2), 3.44 and 3.49 (each 1H, each d, *J* 13.8, 10-H_2), 4.02 (1H, dd, *J* 7.5, 4.6, 2-H); δ_C (75 MHz) 19.9 and 20.4 (C-8 and C-9), 27.2 [C(*C*H_3)$_3$], 26.6, 32.7 and 38.8 (C-3, C-5 and C-6), 42.3 [*C*$(CH_3)_3$], 44.2 (C-4), 47.7 and 48.0 (C-1 and C-7), 53.7 (C-10), 67.2 (C-2), 179.4 [*C*OC$(CH_3)_3$]; *m/z* (CI) 300 (MH^+, 82%), 256 (16), 236 (42), 216 (44), 135 (100) and 57 (98). The middle fraction gave the required (1*S*,2*R*)-*N*-acetylbornane-10,2-sultam **41** (0.289 g, 75% based on sodium acetate) as a white solid; mp 131–132 °C (MeOH); (lit.,[44] 130–132 °C); $[\alpha]_D$ −116.1 (*c* 3.05 in CH_2Cl_2) [lit.,[44] −161.7 (*c* 3.20 in CH_2Cl_2)]; ν_{max} ($CHCl_3$)/cm^{-1} 1694 (CO); δ_H (300 MHz) 0.97 and 1.16 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.40 (2H, m), 1.90 (3H, m), 2.12 (2H, m, 3-H_2, 4-H, 5-H_2 and 6-H_2), 2.40 (3H, s, $COCH_3$),

3.44 and 3.51 (2 × 1H, 2 × d, *J* 14.0, 10-H_2), 3.85 (1H, dd, *J* 7.7, 5.2, 2-H); δ_C (75 MHz) 19.9 and 20.8 (C-8 and C-9), 23.2 ($CO\mathit{C}H_3$), 26.4, 32.8 and 38.4 (C-3, C-5 and C-6), 44.7 (C-4), 47.8 and 48.4 (C-1 and C-7), 52.8 (C-10), 65.2 (C-2) and 168.6 ($\mathit{C}OCH_3$); *m*/*z* (CI) 258.1159 (MH^+, 100%. $C_{12}H_{20}NO_3S$ requires 258.1164), 214 (28), 135 (93), 109 (24) and 83 (36). The most polar fraction gave recovered auxiliary **39** (25.2 mg) as a white solid.

The above reaction was repeated using sodium [1-^{13}C]acetate (2.498 g, 30.1 mmol) and (1*S*)-bornane-10,2-sultam **39** (7.127 g, 33.1 mmol). Purification by column chromatography eluting with 0–50% ethyl acetate–petrol (40–60) gave three main fractions. The least polar fraction gave **42** (0.32 g, 3.2% based on **39**) as a white solid. Data as previously given. The middle fraction gave the required (1*S*,2*R*)-*N*-([1-^{13}C]acetyl)bornane-10,2-sultam **41** (4.95 g, 64% based on sodium [1-^{13}C]acetate) as a white solid; δ_H (300 MHz) 0.97 and 1.16 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.38 (2H, m, 5-*H*H and 6-*H*H), 1.90 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.12 (2H, m, 3-H_2), 2.41 (3H, d, *J* 6.5, $^{13}COCH_3$), 3.43 and 3.51 (each 1H, each d, *J* 13.7, 10-H_2), 3.85 (1H, dd, *J* 7.5 and 5.1, 2-H); δ_C (75 MHz) 19.9 and 20.8 (C-8 and C-9), 23.2 (d, *J* 51.5, $^{13}CO\mathit{C}H_3$), 26.4 and 32.8 (C-5 and C-6), 38.4 (C-3), 44.6 (C-4), 47.7 and 48.3 (C-1 and C-7), 52.8 (C-10), 65.2 (C-2) and 168.6 (^{13}CO enriched); *m*/*z* 258 (M^+, 9%), 243 (3), 215 (8), 151 (53), 134 (86) and 84 (100). The most polar fraction gave recovered **39** (2.19 g) as a white solid.

The above reaction was repeated using sodium [1,2-$^{13}C_2$]-acetate (2.5048 g, 29.8 mmol) and (1*S*)-bornane-10,2-sultam **39** (7.081 g, 32.7 mmol). Purification by column chromatography eluting with 0–50% ethyl acetate–petrol (40–60) gave **42** (2.32 g, 24% based on **39**) followed by the required (1*S*,2*R*)-*N*-([1,2-$^{13}C_2$]-acetyl)bornane-10,2-sultam **41** (5.81 g, 75% based on sodium [1,2-$^{13}C_2$]acetate) as a white solid; δ_H (300 MHz) 0.97 and 1.16 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.38 (2H, m, 5-*H*H and 6-*H*H), 1.90 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.12 (2H, m, 3-H_2), 2.40 (3H, dd, *J* 130.1 and 6.6, $^{13}CO^{13}CH_3$), 3.43 and 3.51 (each 1H, each d, *J* 13.8, 10-H_2), 3.85 (1H, dd, *J* 7.6 and 5.4, 2-H); δ_C (75 MHz) 19.9 and 20.8 (C-8 and C-9), 23.2 (d, *J* 51.5, $^{13}CO^{13}\mathit{C}H_3$), 26.4 and 32.8 (C-5 and C-6), 38.4 (C-3), 44.6 (C-4), 47.7 (C-1 or C-7), 48.3 (d, *J* 2.5, C-1 or C-7), 52.7 (C-10), 65.1 (C-2) and 168.6 (d, *J* 51.5, ^{13}CO); *m*/*z* 259 (M^+, 9%), 244 (3), 215 (7), 134 (100) and 108 (76). The most polar fraction gave recovered **39** (0.1 g) as a white solid.

(1*S*,2*R*)-*N*-[(3′*RS*)-3′-Hydroxybutanoyl]bornane-10,2-sultam 43

Titanium tetrachloride (1 M solution in dichloromethane, 7.0 mL, 7.0 mmol) was added slowly to (1*S*,2*R*)-*N*-acetylbornane-10,2-sultam **41** (0.9 g, 3.5 mmol) in dry dichloromethane (5 mL) at −78 °C in an oven dried flask under nitrogen. After 10 minutes, diisopropylethylamine (1.2 mL, 7.0 mmol) was added dropwise and the reaction mixture was stirred for 0.5 h at −78 °C. Acetaldehyde (~2.0 mL, ~35 mmol) was added and the reaction mixture was stirred at −78 °C for 6 h, before allowing to warm to rt overnight. Saturated aqueous ammonium chloride (40 mL) was added and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with distilled water (20 mL), dried ($MgSO_4$) and the solvents removed *in vacuo*. Purification by column chromatography eluting with 0–40% ethyl acetate–petrol (40–60) gave the alcohols **43** (0.878 g, 83%) as a 3:1 mixture of diastereomers by ^{1}H-NMR spectroscopy; mp 151–154 °C (from MeOH) (Found: C, 55.91; H, 7.55; N, 4.55; S, 10.32. $C_{14}H_{23}NO_4S$ requires C, 55.81; H, 7.64; N, 4.65; S, 10.63%); ν_{max} ($CHCl_3$)/cm^{-1} 3544 (OH) and 1682 (C=O); δ_H (300 MHz) 0.98 and 1.15 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.25 (3H, d, *J* 6.4, 4′-H_3), 1.40 (2H, m), 1.91 (3H, m), 2.13 (2H, m, 3-H_2, 4-H, 5-H_2 and 6-H_2), 2.79 (1H, dd, *J* 17.0, 9.0, 2′-*H*H of major diastereomer), 2.79 (1H, dd, *J* 17.0 and 9.0, 2′-*H*H of minor diastereomer), 2.92 (1H, dd, *J* 17.0, 3.0, 2′-H*H* of major diastereomer), 2.94 (1H, dd, *J* 17.0, 3.0, 2′-H*H* of minor diastereomer), 3.12 (1H, br s, OH), 3.44 and 3.52 (2 × 1H, 2 × d, *J* 13.9, 10-H_2), 3.88 (1H, dd, *J* 7.7, 5.1, 2-H), 4.27 (1H, m, 3′-H); δ_C (75 MHz) 19.8 and 20.8 (C-8 and C-9), 22.3 (C-4′), 26.4, 32.8 and 38.4 (C-3, C-5 and C-6), 43.7 (C-2′), 44.6 (C-4), 47.8 and 48.5 (C-1 and C-7), 52.9 (C-10), 64.2 (C-3′), 65.1 (C-2), 171.4 (C-1′); *m*/*z* (CI) 302.1422 (MH^+, 29%. $C_{14}H_{24}NO_4S$ requires 302.1426), 284 (37), 258 (25), 216 (100), 135 (63).

The above reaction was repeated using (1*S*,2*R*)-*N*-([1-^{13}C]-acetyl)bornane-10,2-sultam **41** (2.50 g, 9.69 mmol) giving the [1′-^{13}C]alcohols **43** (2.76 g, 94%) as a 3:1 mixture of diastereomers by ^{1}H-NMR spectroscopy.

The above reaction was repeated using (1*S*,2*R*)-*N*-([1,2-$^{13}C_2$]acetyl)bornane-10,2-sultam **41** (3.0 g, 11.6 mmol) giving the [1′,2′-$^{13}C_2$]alcohols **43** (3.29 g, 94%) as a 3:1 mixture of diastereomers by ^{1}H-NMR spectroscopy.

(1*S*,2*R*)-*N*-[(*E*)-Crotonyl]bornane-10,2-sultam 45

Pyridine (1 mL) and acetic anhydride (0.5 mL) were added to (1*S*,2*R*)-*N*-[(3′*RS*)-3′-hydroxybutanoyl]bornane-10,2-sultam **43** (100 mg, 0.33 mmol) in dichloromethane (2 mL) at rt. The reaction mixture was left standing at rt overnight, before quenching with saturated aqueous sodium hydrogen carbonate (20 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The organic phase was washed with hydrochloric acid (2 M, 30 mL), saturated aqueous brine (30 mL), dried ($MgSO_4$) and the solvents removed *in vacuo*. The crude product (0.21 g) was dissolved in dichloromethane (4 mL), DBU (0.27 g, 1.61 mmol, 1.2 eq.) was added and the reaction mixture was left standing overnight. The reaction mixture was acidified with hydrochloric acid (2 M, 10 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with saturated aqueous brine (20 mL), dried ($MgSO_4$) and the solvents removed *in vacuo*. Purification by column chromatography eluting with 10–20% ethyl acetate–petrol (40–60) gave the title compound **45** (77 mg, 81%) as a white solid. Spectroscopic data as previously reported.[45]

The above reaction was repeated using (1*S*,2*R*)-*N*-[(3′*RS*)-3′-hydroxy[1-^{13}C]butanoyl]bornane-10,2-sultam **43** (5.8 g, 16.6 mmol). Purification by column chromatography eluting with 10–20% ethyl acetate–petrol (40–60) gave (1*S*,2*R*)-*N*-[(*E*)-[1-^{13}C]crotonyl]bornane-10,2-sultam **45** (4.64 g, 85%) as a white solid; δ_H (300 MHz) 0.98 and 1.18 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.40 (2H, m, 5-*H*H and 6-*H*H), 1.89 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.13 (2H, m, 3-H_2), 1.94 (3H, ddd, *J* 7.0, 1.7, 1.0, 4′-H_3), 3.45 and 3.52 (2 × 1H, 2 × d, *J* 13.8, 10-H_2), 3.93 (1H, m, 2-H), 6.59 (1H, ddq, *J* 15.0, 6.1, 1.7, 2′-H), 7.11 (1H, dquintet, *J* 15.0, 7.0, 3′-H); δ_C (75 MHz) 18.3 (d, *J* 7.4, C-4′), 19.9 and 20.8 (C-8 and C-9), 26.5 and 32.8 (C-5 and C-6), 38.5 (C-3), 44.6 (C-4), 47.7 and 48.4 (C-1 and C-7), 53.1 (C-10), 65.1 (C-2), 122.2 (d, *J* 66.4, C-2′), 146.1 (C-3′), 164.0 (^{13}C-1′); *m*/*z* 284 (M^+, 17%), 269 (3), 220 (15), 205 (32), 108 (18) and 70 (100).

The above reaction was repeated using (1*S*,2*R*)-*N*-[(3′*RS*)-3′-hydroxy[1,2-$^{13}C_2$]butanoyl]bornane-10,2-sultam **43** (6.36 g, 21.0 mmol). Purification by column chromatography eluting with 10–20% ethyl acetate–petrol (40–60) gave (1*S*,2*R*)-*N*-[(*E*)-[1,2-$^{13}C_2$]crotonyl]bornane-10,2-sultam **45** (5.17 g, 86%) as a white solid; δ_C (300 MHz) 0.98 and 1.18 (2 × 3H, 2 × s, 8-H_3 and 9-H_3), 1.41 (2H, m, 5-*H*H and 6-*H*H), 1.88 (3H, m, 4-H, 5-H*H* and 6-H*H*), 1.94 (3H, tdd, *J* 7.0, 1.7, 1.0, 4′-H_3), 2.12 (2H, m, 3-H_2), 3.45 and 3.52 (2 × 1H, 2 × d, *J* 13.8, 10-H_2), 3.93 (1H, m, 2-H), 6.59 (1H, dddq, *J* 161.2, 15.0, 6.1, 1.7, 2′-H), 7.11 (1H, m, 3′-H); δ_C (75 MHz) 18.3 (d, *J* 8.7, C-4′), 19.9 and 20.8 (C-8 and C-9), 26.5 and 32.8 (C-5 and C-6), 38.5 (C-3), 44.7 (C-4), 47.7 (C-1 or C-7), 48.4 (d, *J* 2.5, C-1 or C-7), 53.1 (C-10), 65.1 (C-2), 122.3 (d, *J* 65.8, ^{13}C-2′), 146.1 (d, *J* 71.3, C-3′), 164.0

(d, J 65.8, ^{13}C-1′); m/z 285 (M^+, 26%), 270 (4), 257 (5), 206 (55), 135 (38) and 71 (100).

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