Preparation and Characterization of [5-^{13}C]-(2*S*,4*R*)-Leucine and [4-^{13}C]-(2*S*,3*S*)-Valine − Establishing Synthetic Schemes to Prepare Any Site-Directed Isotopomer of L-Leucine, L-Isoleucine and L-Valine

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In this paper a chemo-enzymatic method has been developed that gives access to any isotopomer of the essential amino acids isoleucine and valine. The method gives the correct introduction of the second chiral center in (2*S*,3*S*)-isoleucine and allows for discrimination between the two prochiral methyl groups in valine as shown by the preparation of (2*S*,3*S*)-[4-^{13}C] valine. For the preparation of (2*S*)-leucine in any isotopomeric form, the O'Donnell method to prepare optically active amino acids has been used. The protected glycine scaffold used in this method has been prepared by a strategy that allows access to any isotopomeric form. The preparation of [5-^{13}C]-(2*S*,4*R*)-leucine shows that the O'Donnell method in combination with the Evans method to obtain chiral 2-methylpropyl iodide leads to a good discrimination between the two prochiral methyl groups. The O'Donnell strategy for the preparation of α-amino acids is preferred over other methods since the reaction conditions are mild, the chiral auxiliary can be easily recovered and the optically active product can be easily separated. For the preparation of isotopically enriched valine and isoleucine the O'Donnell method is not suitable, because the alkyl substituents involved have a secondary halide substituent which is sterically too hindered to give an effective reaction with the protected glycine.

Introduction

The primary structures of all human proteins are now available with the completion of the human genome project[1]. Very rapidly, in the post-genomic era, the total genomes of a plethora of other organisms are also becoming available, as are mutants that lead to malfunctioning or nonfunctioning proteins leading to genetic diseases. Furthermore, efficient biotechnological procedures are available to obtain (membrane) proteins using these genetic codes.[2,3] The fundamental challenge now is to study the chemical processes of these proteins involving (bio)macromolecules without perturbation in the native states at the atomic level in time scales ranging from femtoseconds up to days. Nature provides us with the ultimate probe by stable isotopes. Isotopes combine the same chemistry with different physical properties.[4] Study of a system with site-directed isotope labeling with a high incorporation allows the determination of the whole force field by vibrational techniques such as FT IR spectroscopy and (resonance) Raman spectroscopy based on the difference in isotopic mass.[5−7] These techniques probe, for instance, the electron density in chemical bonds of the isotope-labeled molecule. Other spectroscopic methods are solution and solid-state magic angle spinning (MAS) NMR spectroscopy, which probe the electron density at the atoms. These techniques allow the establishment of electronic charges in the atoms, protonation states, and configurations and conformations around bonds of the stable isotopically labeled molecule.[8,9] Even bond lengths can be resolved with picometer resolution and torsion angles can be determined along selected bonds in a protein system.

Comparison of the structural parameters obtained by these techniques for intermediate I and intermediate I + 1 in the biochemical process of the studied system provides functional information, such as changes in protonation states, bond lengths, configuration and conformation around bonds on the time scale involved.[7,8] When sufficient structural and functional information at the atomic level of the native form has been obtained, a whole new dimension can be attained by studying in a similar fashion systems with mutations in the protein chain and systems with rationally designed chemical changes in the cofactors.[9,10] These studies will lead to an even deeper understanding of the biochemical process. Implementation of the above-mentioned program is now of the utmost urgency. Without this program, the increasingly available genetic information cannot be translated into the required structural and functional information that will lead to the expected quantum leap in the understanding of the various processes in human (and animal) health and diseases and the ex-

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pected rational approach to treat these diseases. Access to the full set of possible site-directed isotopically enriched amino acids up to the uniformly labeled systems is a conditio sine qua non for the proposed structural and fundamental investigations. All uniformly ^{13}C- and ^{15}N-enriched amino acids are commercially available by photosynthetic organisms that are grown in media containing $^{13}CO_2$ and $^{15}NH_3$.[11] However, all other possible site-directed isotopomers of amino acids cannot be obtained by biosynthesis. The only way to obtain access to the whole set of desired isotopomers is by a modular synthetic approach such that one synthetic scheme can give in a rational way any L-amino acid in a set of all isotopomers. The approach may seem Herculean although, in fact, only 20 different amino acids are needed.

In the recent past we have already realized this for 9 of the 20 amino acids[12−15]. These are Glu, Gln, His, Phe, Pro, Ser, Thr, Trp and Tyr. Gly and Ala are commercially available labeled in any position or combination of positions. Of the remaining nine amino acids that are introduced in the protein chain by the translation process in the cell, this paper focuses on the three amino acids with aliphatic side chains, namely valine, leucine and isoleucine. There are indications that the aliphatic side chains of amino acids are involved in protein stabilization through interactions with aromatic residues of Trp, Tyr and Phe in the protein.[16] Previously, no method existed to obtain direct structural information about these interactions, This has now been changed dramatically by the advent of ultra-high-field ^{1}H and ^{13}C solid-state NMR techniques. The first solid-state ^{1}H and ^{13}C chemical shift study of [$^{13}C_{20}$]rhodopsin showed that the biggest shifts are due to the interaction of the chromophore with the aromatic side chains in the active site occuring on the 16-H_3 and 17-H_3 methyl groups of the aliphatic ring of (11*Z*)-retinal. Access to valine, leucine and isoleucine with site-directed ^{13}C enrichment in the methyl groups will allow these interactions to be quantitatively established in any protein at the atomic level by ultra-high-field ^{1}H and ^{13}C NMR techniques. Another motive for the development of synthetic schemes for the synthesis of the full set of ^{13}C isotopomers of valine, leucine and isoleucine is that these three are essential amino acids in human and animal nutrition. These amino acids have to be present in food because animals and humans cannot biosynthesize them. The access to isotopomers will allow nutritional and metabolic studies (metabolomics) in individual humans (and animals) at the physiological level without perturbation, based on a similar study for [$^{13}C_{10}$]-β-carotene which we, together with other groups, recently published.[17] An additional motivation for the preparation of the isotopomers of leucine is that leucine is essential in gene regulation by the formation of leucine zippers and the regulation of protein turnover. The essential role of leucine in these vital processes will also be amenable to study at the atomic level without perturbation via site-directed isotopomers.

However, the most compelling reason to have access to the full set of isotopomers of leucine and isoleucine is that these amino acids are isobaric. This fact is a strong handicap to the development of high-throughput mass spectral analysis as a tool in proteonomics, which is currently the method of choice to analyze the full set of proteins present in the cell. Leucine, isoleucine and hydroxyproline have the same molecular mass, such that in a protein the sequence containing these amino acids can only be easily analyzed when the proteins are formed in media in which these residues differ in isotopic composition.[18,19]

A complication for synthetic schemes to prepare the whole set of isotopomers of these aliphatic amino acids is that both valine (**1**) and leucine (**3**) each have two diastereotopic methyl groups. Their numbers are 4 and 5 for valine and 5 and 6 for leucine (**3**) in Figure 1. If these groups differ in isotope incorporation, chirality is introduced, leading to a diastereotopic mixture. Any synthetic schemes should always be such that a precisely defined chirality will be present.

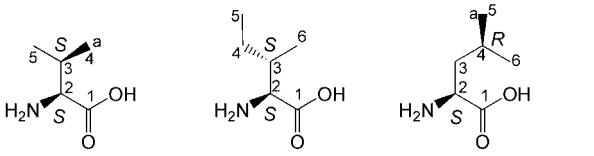


Figure 1. Structures of (2*S*)-valine (**1**), (2*S*)-isoleucine (**2**) and (2*S*)-leucine (**3**); **1a** and **3a** represent the site-directed, ^{13}C-enriched forms

Isoleucine (**2**) has the complication that carbon atom 3 has the (*S*) chirality. Here, also, the synthetic scheme should only lead to the required stereochemistry. The starting point for the development of a synthetic scheme to prepare the full set of isotopomers of valine (**1**) is the fact that in the literature[20] an enzymatic conversion of mesaconic acid into (2*S*,3*S*)-methylaspartic acid has been described, which could then be converted into (2*S*)-valine (**1**). Using this scheme, Kluender et al. were able to prepare just [4-^{13}C]-(2*S*,3*S*)-valine, because their scheme only allowed the preparation of mesaconic acid with ^{13}C enrichment in the methyl group. In this paper we describe the efficient synthesis of all isotopomers of mesaconic acid. For the preparation of (2*S*)-valine (**1**) we optimized the subsequent conversion of (2*S*,3*S*)-methylaspartate into valine (**1**). The synthetic scheme for the isotopomers of (2*S*)-isoleucine (**2**) could be based on a rational modification of the scheme for (2*S*)-valine (**1**). For access to all isotopomers of (2*S*)-leucine (**3**) we devised a strategy based on a synthetic reaction involving optically active accessory materials.[21] For the preparation of isotopomers this leads to a well-defined scheme by which any isotopomer is accessible without question. For (2*S*)-valine (**1**) and (2*S*)-leucine (**3**) the most critical question is a good differentiation in isotopic enrichment in the two diastereotopic methyl groups. In order to establish the efficiency of our scheme in this regard we describe the preparation of [4-^{13}C]-(2*S*,3*S*)-valine and [5-^{13}C]-(2*S*,4*R*)-leucine. In these two systems the efficiency of our schemes

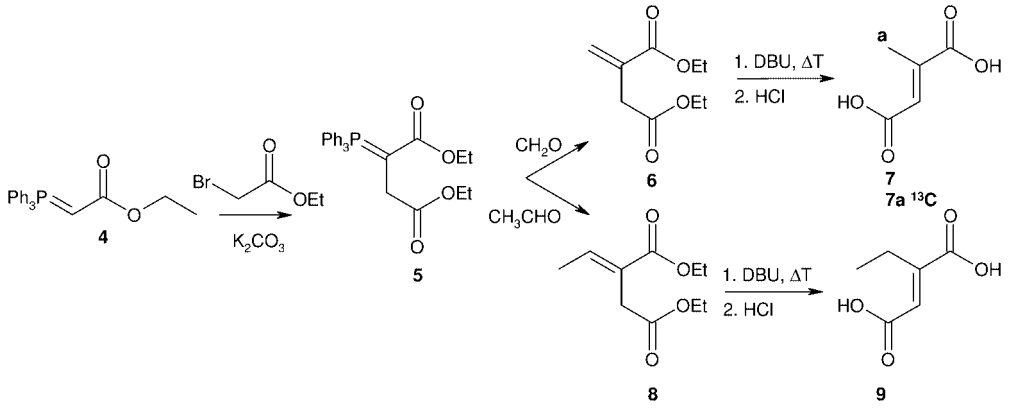
can be easily established using ^{1}H and ^{13}C NMR techniques.

Results and Discussion

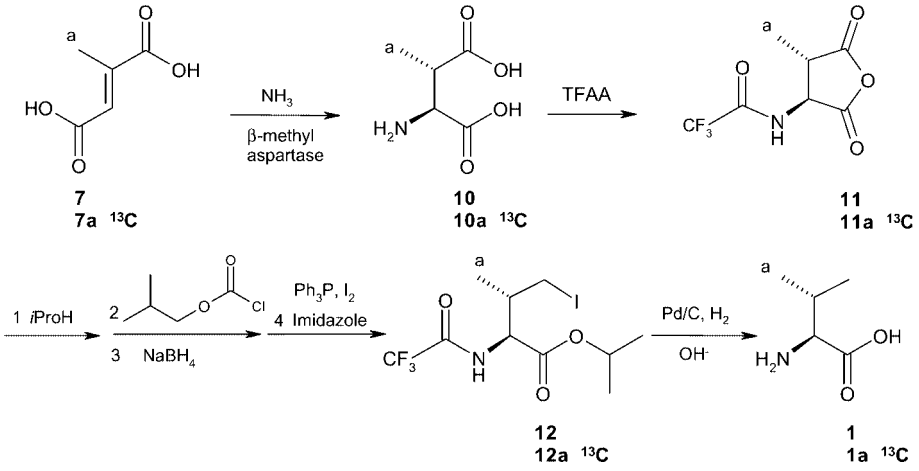
Synthetic Strategy

For the synthesis of the isotopomers of valine we use the scheme starting from mesaconic acid (**7**; Scheme 2) as pioneered by Kluender et al. In order to obtain the full set of isotopomers of valine, the synthesis of mesaconic acid (**7**) which has the complete carbon skeleton of valine (**1**) is essential. Scheme 1 indicates how mesaconic acid (**7**) can be prepared starting from the phosphorane **4**, which can be obtained in a few steps from acetic acid as previously described by our group.[22] This scheme was first optimized by carrying out all the reactions with reagents with natural isotope abundance. Treatment of **4** with ethyl bromoacetate in the presence of solid K_2CO_3 gives the phosphorane **5**. Reaction of **5** with formaldehyde gives the itaconic diester **6**. Upon treatment with DBU the *exo* double bond isomerizes to the main chain to give a mixture of citraconic and mesaconic diesters. Heating with concentrated hydrochloric acid hydrolyzed the ester groups and effected isomerization of the double bond to give, within experimental error, only the thermodynamically more stable *trans* form, mesaconic acid (**7**). It is clear that the number of synthons and steps used is kept to a minimum. All synthons are commercially available in all isotopomeric forms. The reactions in these schemes give simple access to pure mesaconic acid in any site-directed isotopic form. The yield from **4** to **7** is 52 %.

Scheme 2 shows the conversion of mesaconic acid (**7**) into (2*S*)-valine (**1**) with chiral discrimination between the diastereotopic methyl groups. The first step in the stereoselective conversion of **7** into (2*S*,3*S*)-3-methylaspartic acid (**10**) was carried out in aqueous solution by β-methyl aspartase, which we isolated from *Clostridium tetanomorphum*.[23] The yield after one pass was 65 %; recovery of the starting material and two further reaction cycles gave a total yield of 92 %. Introduction of a ^{15}N label is possible by using $^{15}NH_4Cl$ as the nitrogen source. Methylaspartic acid (**10**) can be converted into valine (**1**) by selectively reducing the γ-carboxyl group. In order to make a distinction possible between the two carboxylic acid moieties the C-1 carboxylic acid was converted into an ester. Addition of trifluoroacetic anhydride in THF to the amino diacid resulted in the protection of the amine group by formation of a trifluoroacetamide function and formation of the cyclic anhydride **11**. Alcoholysis of the anhydride with 2-propanol at −5 °C gave



Scheme 1. Synthesis of the carbon skeleton of valine (**1**) and isoleucine (**2**)

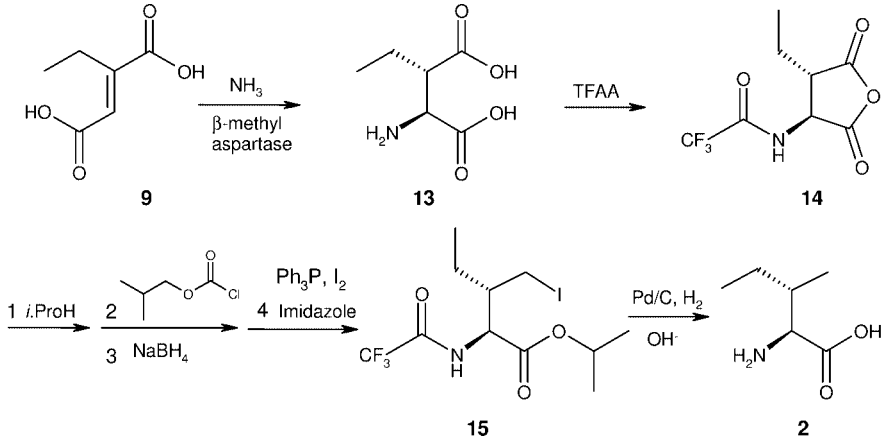


Scheme 2. Synthesis of (2*S*,3*S*)-valine (**1a**) starting from achiral mesaconic acid (**7**)

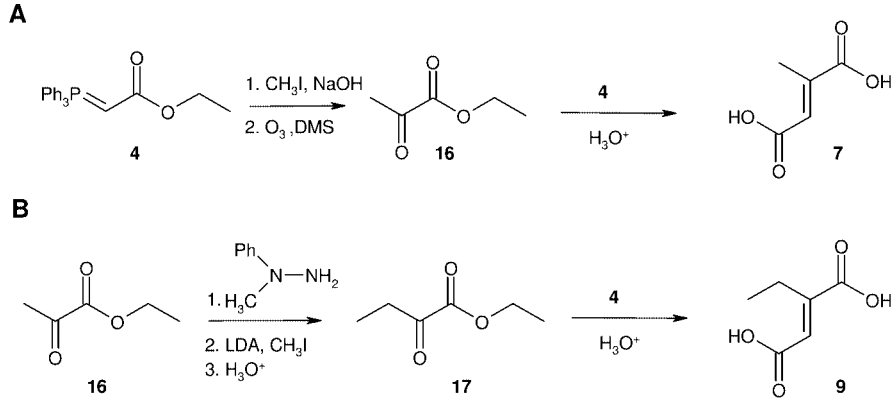
exclusively the C-1 ester[24]. The protection of the C-1 ester leaves the C-4 acid open to reaction with isobutyl chloroformate and subsequent reduction by sodium borohydride. The resulting alcohol was converted into the iodide **12** using elemental iodine and triphenylphosphane. Exchange of the iodine for hydrogen by palladium on coal and hydrogen gas gave the protected (2*S*)-valine (**1**). Deprotection could be accomplished using aqueous base, yielding the desired (2*S*)-valine (**1**). The yield from **7** to **1** is 40 %. The reactions described in Schemes 1 and 2 lead to any site-directed ^{13}C and ^{15}N isotopomer of (2*S*)-valine (**1**) up to the unitary labeled form. To show that this scheme allows the chiral discrimination between the two diastereotopic methyl groups, [4-^{13}C]valine was prepared. Starting from **7a**, [4-^{13}C]-(2*S*,3*S*)-valine (**1a**) could be prepared by the reactions in Scheme in 35 % yield. Spectral analysis showed that the isotope label was specifically and selectively incorporated in the (*S*)-methyl group (vide infra).

Isoleucine

The synthesis of L-isoleucine (**2**) can be carried out in an analogous manner to the synthesis of L-valine (**1**). β-Methyl aspartase, the enzyme that converts the achiral mesaconic acid into the chiral (2*S*,3*S*)-methylaspartic acid is not restricted to one substrate.[25] Fumaric acid derivatives with side chains up to the length of a propyl group fit the binding pocket of the enzyme. Moreover, 2-chloro- and 2-fluorofumaric acid can be converted into the corresponding haloaspartic acid analogues. This allows the extension of the methods in Schemes 1 and 2 to the synthesis of the isotopomers of (2*S*,3*S*)-isoleucine (**2**). Using 2-ethylfumaric acid (**9**) as a substrate, (2*S*,3*S*)-ethylaspartic acid (**13**) could be prepared and converted into L-isoleucine (**2**) by the transformations in Scheme 3. The enzymatic substrate **10** was obtained by treating acetaldehyde with **5** followed by isomerization of the double bond with DBU. Hydrolysis of the esters with concentrated HCl gave the free diacid while at the same time effecting the isomerization of ethylmaleic diacid to the desired 2-ethylfumaric acid (**9**). Enzymatic conversion of the substrate gave after three passes a 90 % yield of ethylaspartic acid (**13**). The chirality of the two stereocenters in the isoleucine skeleton is determined in the enzymatic step. The formation of the (*S*) configuration at C-2 leads to the (2*S*)-amino acid, while the (*S*) conformation at C-3 will result in the correct chirality at the branching point of the isoleucine side chain. Following the chain of reactions shown in Scheme 3, **13** could be converted into (2*S*)-isoleucine (**2**). The reactions in Schemes 2 and 3 lead to any ^{13}C, ^{15n} isotopomer of (2*S*)-isoleucine (**2**). Because any atom in isoleucine (**2**) has a well-defined source and no complications arise from prochiral groups, there is no need to further check this scheme.



Scheme 3. Synthesis of (2*S*,3*S*)-isoleucine (**2**) starting from achiral 2-ethylfumaric acid (**9**)



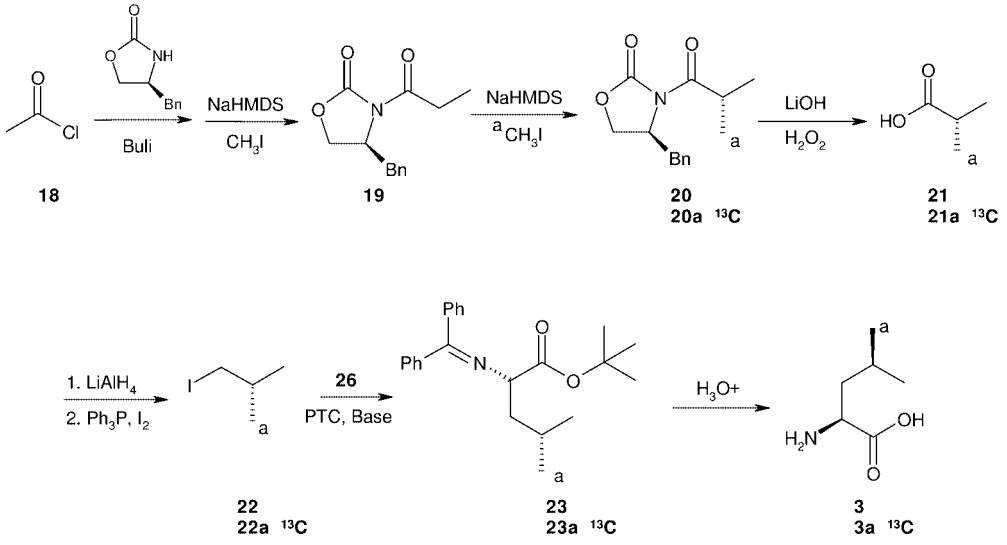
Scheme 4. Alternative synthesis of mesaconic acid (**7**) and 2-ethylfumaric acid (**9**)

Before we prepared mesaconic acid (**7**) and 2-ethylfumaric acid (**9**) according to Scheme 1, we explored the reactions in Scheme 4. Mesaconic acid (**7**; Scheme 4, A) could be prepared in the following fashion. Phosphorane **4** was treated with methyl iodide and then with base. In this way a substituted phosphorane was obtained, which could be converted into ethyl pyruvate (**16**) by treatment with ozone and subsequent reduction of the product with dimethyl sulfide. Ethyl pyruvate (**16**) reacted with the phosphorane **4** to give an (*E*)/(*Z*) mixture of mesaconic esters, which on aqueous HCl treatment gave within experimental error the pure (*E*)-mesaconic acid (**7**). A similar conversion of **4** with ethyl iodide gives **17** in very low yields, owing to the competing reaction of formation of ethene by elimination of HI. Compound **17** has been prepared by first converting ethyl pyruvate (**16**) into the corresponding methyl phenyl hydrazone. Subsequent treatment with LDA and S_N2 reaction with CH_3I followed by mild acidic workup gave ethyl 2-oxobutyrate (**17**). The hydrazone protection can give access to a range of pyruvate analogues, if methyl iodide is replaced with other suitable electrophiles. Treatment of **17** with **4** gave an (*E*)/(*Z*) mixture of substituted maleic and fumaric esters. Hydrolysis of the esters and isomerization to the most stable form was effected by refluxing in concentrated hydrochloric acid, giving exclusively 2-ethylfumaric acid (**9**). The synthetic route presented in Scheme 4 enables the synthesis of isotopically enriched **7** and **9** with a carbon label at each position or combination of positions.

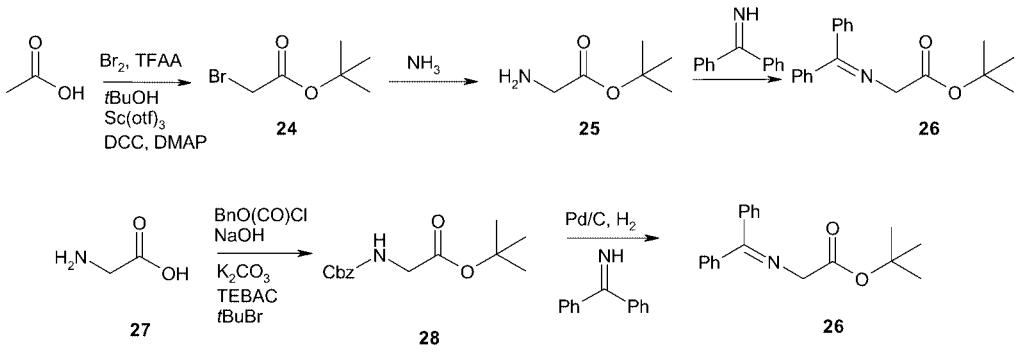
Leucine

Isotopomers of (2*S*)-leucine (**3**) can not be prepared by a chemo-enzymatic method as described for (2*S*)-valine (**1**) and (2*S*)-isoleucine (**2**). Recently, the asymmetric alkylation of *tert*-butyl *N*-(diphenylmethylene)glycinate (**26**) under cinchona alkaloid derived phase-transfer catalytic conditions has been optimized.[21,26] The reaction conditions are mild with a high yield of α-amino acids and very high enantiomeric excess. Additional advantages of this method are that the base, the chiral catalyst and the *tert*-butyl *N*-(diphenylmethylene)glycinate (**26**) are all commercially available. In order to test the procedure we treated commercial 2-methylpropyl iodide with the protected glycine ester **22**. Reaction under phase-transfer conditions with BTPP [BTPP = (*tert*-butylimino)tripyrrolidinophosphorane] as base in dichloromethane at −50 °C gave 76 % of the protected leucine **23** which after deprotection gave (2*S*)-leucine (**3**) (95 % *ee*, after crystallization 99 % *ee*). It is clear that for the preparation of the full set of isotopomers of leucine (**3**) both reactants, namely 2-methylpropyl iodide (**22**) and the protected glycine derivative **23**, have to be available as the full set of well-defined isotopomers. The most critical requirement is to have the diastereotopic methyl groups in exactly defined ^{13}C-enriched form. We started to effect the latter requirement first. Scheme 5 indicates how via the Evans template a propionic side chain can be converted into a ^{13}C-isobutyric side chain which by hydrolysis, $LiAlH_4$ reduction and subsequent iodination can be converted into [^{13}C]-(2*S*)-methylpropyl iodide with 76 % enantiomeric excess. This incorporation is sufficient for our present purpose. However, it can be improved by using the Evans template with an isopropyl group.[27] This latter synthon is not commercially available, in contrast to the benzyl system. The propionyl side chain on the Evans template can be prepared in all isotopomeric forms, starting from acetyl chloride (commercially available in all isotopically enriched combinations), which after coupling with the template can be alkylated with CH_3I to give the propionic side chain.

The second step in the strategy is to prepare the complete set of ^{15}N and ^{13}C isotopomers of the protected glycine reagent. The introduction of the ^{13}C isotopes is straightforward. 2-Bromoacetic acid is commercially available as the set of all possible ^{13}C isotopomers. It is converted into the required protected glycine derivative **26** by the reactions depicted in Scheme 6. Esterification with *tert*-butyl alcohol gave *tert*-butyl 2-bromoacetate. The bromide function can be substituted into a $^{14}NH_2$ group by reaction with liquid



Scheme 5. Synthesis of (2*S*)-leucine (**3**)



Scheme 6. Synthesis of the protected glycine backbone

ammonia, after which the resulting *tert*-butyl glycine is treated with benzophenone imine to give **26** in all the possible ^{13}C isotopomers.

A similar introduction of ^{15}N is prohibitively expensive, however. Therefore, the synthesis of ^{15}N-enriched protected glycine derivative **26** is carried out starting from glycine itself (which is commercially available in all combinations of ^{13}C and ^{15}N enrichment). Treatment of glycine with benzyloxycarbonyl chloride (Cbz) to protect the amino function made it possible to convert the acid group into a *tert*-butyl ester by treating it with *tert*-butyl bromide in the presence of K_2CO_3 and benzyltriethylammonium chloride as the phase-transfer catalyst. Removal of the Cbz protecting group was effected by reduction with palladium on coal and hydrogen gas, which allowed introduction of the benzophenone imine group on the liberated amino function, giving **26**. Having accomplished the preparation of the full set of isotopomers of the protected glycine synthon together with the full set of different isotopomers of 2-methylpropyl iodide, we have access to the full set of all isotopomers of (2*S*)-leucine (**3**). As far as we know leucine is the first α-amino acid for which a synthetic scheme has been based on the O'Donnell strategy to obtain access to the full set of isotopomers.

Although we realized that the O'Donnell strategy is optimized for the preparation of optically active α-amino acids in which the side chain derives from a primary alkyl group, we treated the protected glycine ester **22** with 2-iodopropane to test if the system would also work with a side chain derived from secondary alkyl groups, such as occur in valine (**1**) and isoleucine (**2**). The best result we obtained was 7 % of (2*S*)-valine (**1**) only. The yield of valine (**1**) prepared in this way is too low to be a good substitute for the preparation of (2*S*)-valine (**1**) and (2*S*)-isoleucine (**2**) by a chemo-enzymatic method as we describe in this paper. An experiment with 4-chlorobenzaldimine as the amino protecting group and the chiral quaternary ammonium catalyst devised by Ooi et al.,[28] both chosen for the decreased steric hindrance, gave similar yields, and the literature shows no case of successful alkylation with a secondary halide using the O'Donnell method.

We have previously prepared the full isotopomeric set of the glycine part in the bis(lactim) ether of D-valine-glycine[29]. This is the basic system of the Schöllkopf method for the preparation the full isotopomeric set of α-amino acids. We are now in a position to compare these methods. It is clear that the O'Donnell method for the preparation of optically active amino acids is superior both because of milder reaction conditions and easy workup of both catalyst and required amino acid, which in the case of the Schöllkopf method is always mixed with the same amount of D-valine which has to be separated with preparative ion-exchange chromatography.

Spectroscopic Identification

The samples of (2*S*)-valine (**1**), (2*S*)-isoleucine (**2**) and (2*S*)-leucine (**3**) prepared by the methods described in this paper all have the analytic characteristics of authentic samples. ^{1}H and ^{13}C NMR spectroscopy is the method of choice to establish the location and amount of ^{13}C incorporation in a site-directed ^{13}C-enriched system. In Figure 2 the 400-MHz ^{1}H NMR spectra of [4-^{13}C]-(2*S*,3*R*)-valine (2A) and (2*S*)-valine (2B) are reproduced. The large 125.1-Hz splitting in the 4-H$_3$-(*R*) peak induced by the ^{13}C incorporation is obvious. From the intensities it is clear that the incorporation has to be high; however, the precise incorporation cannot be obtained from the spectra owing to overlap of the peaks of the now enriched (3*R*) group and the (3*S*) methyl group. It is also clear that the 2-H, 3-H and 4-H$_3$-(*S*) signals show additional splitting due to long-range $J_{C,H}$ coupling with [4-^{13}C] (4.9 Hz, 4.4 Hz, 5.4 Hz, respectively). Figure 2 also reproduces the 400-MHz spectra of (2*S*,3*S*)-isoleucine prepared by our method (2C) and commercial (2*S*/*R*,3*S*)-isoleucine (2D). The spectrum of the (2*S*,3*S*) material shows within experimental error the signals of the correct material only, which has the same characteristics as commercial 99 % (2*S*,3*S*)-isoleucine. When compared to a sample of commercial (2*S*/*R*,3*S*)-isoleucine the doubling of the peaks in the (2*S*/*R*,3*S*)-isoleucine spectrum due to the presence of two diastereotopic molecules can be clearly seen. This leads us to conclude that (2*S*,3*S*)-isoleucine can be prepared selectively using the method described before (vide supra).

In Figure 3 the 400-MHz ^{1}H NMR spectra of [5-^{13}C]-(2*S*,4*R*)-leucine (3A) and natural-abundance (2*S*)-leucine (3B) are reproduced. The large 125.3-Hz splitting in the 5-H$_3$ (*R*) peak induced by the ^{13}C incorporation is obvious. From the intensities it is clear that the label incorporation

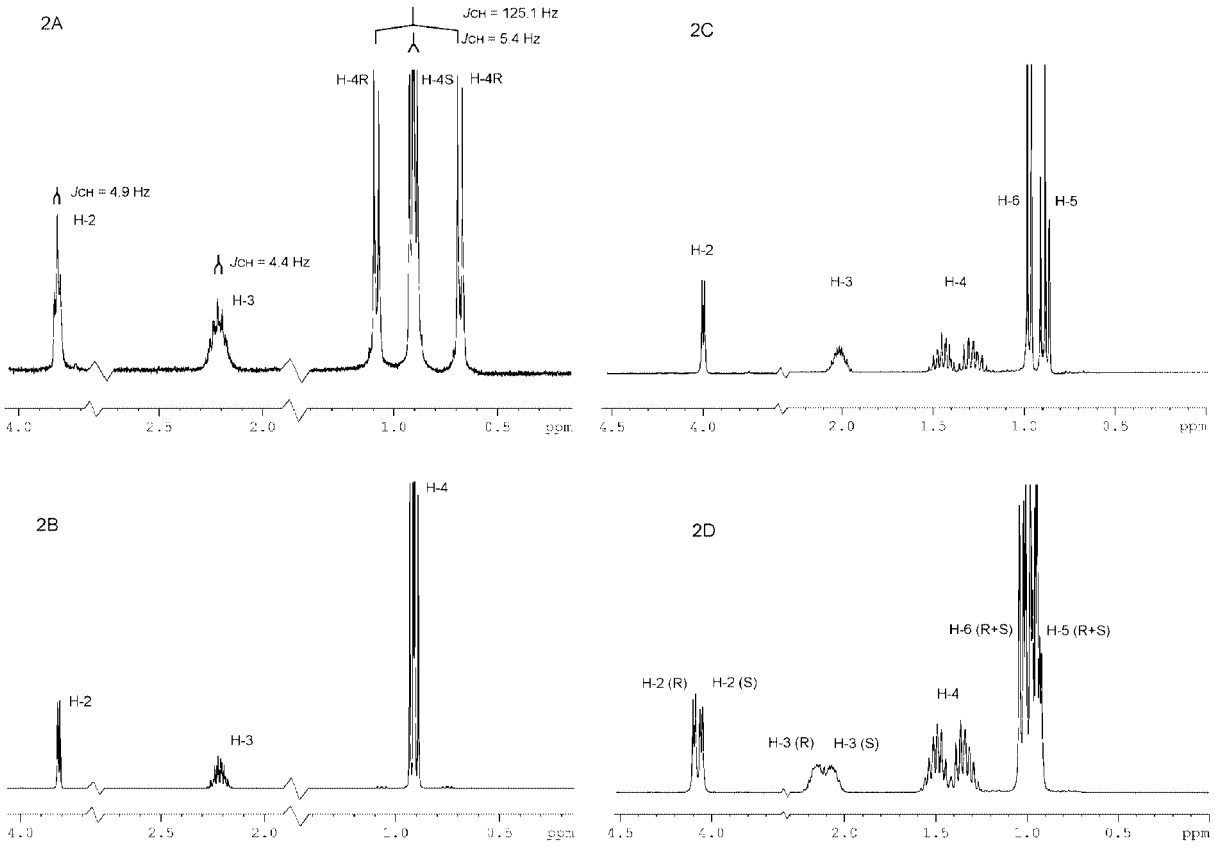


Figure 2. ^{1}H (400 MHz) spectra of [4-^{13}C]-(2*S*,3*S*)-valine (2A), natural-abundance (2*S*)-valine (2B), (2*S*,3*S*)-isoleucine (2C) and (2*S*/*R*,3*S*)-isoleucine (2D)

is divided between C-5*R* (major) and C-5*S* (minor). Decoupling experiments show that the precise ratio of isotope incorporation between C-5*R* and C-5*S* is 8.5:1. It is also clear that the 5-H_3-(*S*) signal shows additional splitting due to long-range $^{3}J_{C,H}$ coupling (5.7 Hz) with [5-^{13}C] (*R*).

The inequivalence of the prochiral methyl groups in valine and leucine also shows itself in the ^{13}C spectra (Figure 3, C and D). Not only can separate peaks be discerned for each methyl group, but the incorporation of a stable ^{13}C isotope makes it clear that the synthetic schemes are selective, as can be witnessed by the selective magnification of the [4-^{13}C]-(3*S*) methyl signal of valine and the [5-^{13}C]-(4*R*) methyl signal of leucine: [^{13}C]valine shows a large peak at $\delta = 17.2$ ppm in the ^{13}C NMR spectrum, while the peak at $\delta = 17.8$ ppm shows no significantly increased intensity (Figure 3), in agreement with literature. For [5-^{13}C]-L-leucine, we expect a ratio of 8.5:1 between the [5-^{13}C]-(4*R*) and -(4*S*) peaks, due to the 8.5:1 (*R*)/(*S*) ratio in the chiral alkylation via the Evans template **18**. Figure 3 (D) shows an increased intensity for both the [5-^{13}C]-(4*R*) and -(4*S*) signals, with the former one having a much higher intensity (8.5:1), as expected. In both 3C and 3D a strong $^{1}J_{C,C}$ coupling between the ^{13}C label and the adjacent C atom can be seen. The splitting is 35.5 Hz in the case of valine and 34.5 Hz in the case of leucine.

Conclusion

Schemes have been successfully developed which allow the selective isotope labelling at each position or any combination of positions for (2*S*)-leucine (**3**), (2*S*)-isoleucine (**2**) and (2*S*)-valine (**1**). To prove that our schemes give the desired selectivity in discriminating between the prochiral methyl groups of leucine and valine, [4-^{13}C]-(2*S*,3*S*)-L-valine and [5-^{13}C]-(2*S*,4*R*)-L-leucine have been prepared in good yield and high enantiomeric excess. Spectral analysis of the products and comparison with literature data proved the validity and specificity of the schemes. For the preparation of (2*S*)-leucine (**3**) in any stable isotopically enriched form a strategy has been developed based on the O'Donnell method for the preparation of optically active α-amino acids. The protected glycine scaffold that is central to the O'Donnell method can be obtained in all required isotopically labelled forms. Access to this isotopically labelled synthon will allow efficient schemes to be developed for the preparation of the remaining five α-amino acids that are introduced by translation processes into proteins. The preferred use of the O'Donnell strategy in the cases where amino acids have been earlier prepared by the Schöllkopf method is expected owing to the easy recovery of the optically active catalyst, the milder reaction conditions and

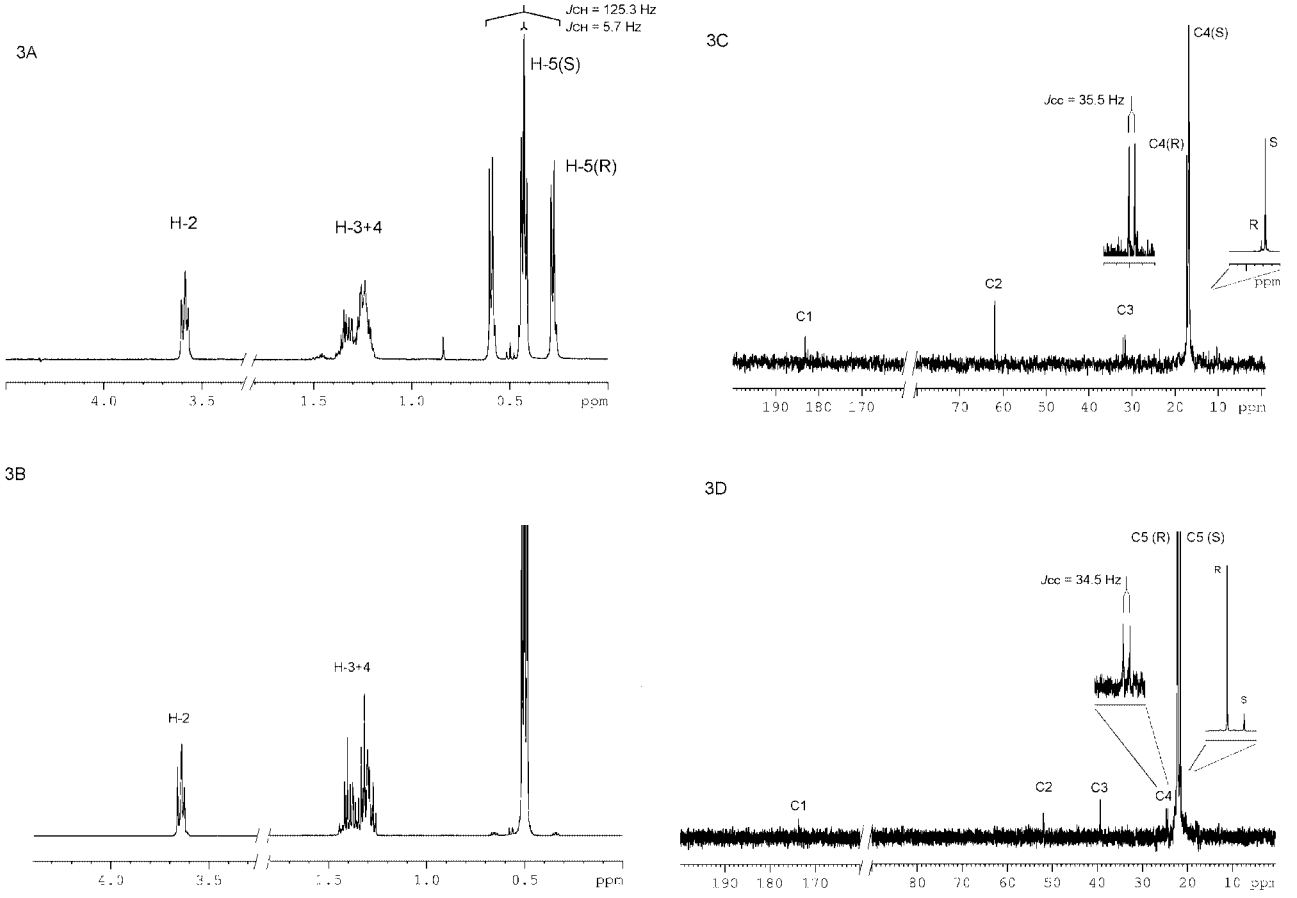


Figure 3. ^{1}H (400 MHz) spectra of [5-^{13}C]-(2*S*,4*R*)-leucine (3A), natural-abundance (2*S*)-leucine (3B) and the ^{13}C (100 MHz) spectra of [4-^{13}C]-(2*S*,3*S*)-valine (3C) and [5-^{13}C]-(2*S*,4*R*)-leucine (3D)

the fact that the final product is easily obtained without the difficult HPLC separation from D-valine that is necessary with the Schöllkopf method.

Experimental Section

General Remarks: ^{1}H NMR spectra were recorded with Jeol FX-200 and Bruker DPX-300 spectrometers, using tetramethylsilane (TMS: δ = 0 ppm), water (H_2O: δ = 4.8 ppm) or 3-(trimethylsilyl)-tetradeuteriopropionic acid (TSP: δ = 0) as internal standards. ^{13}C noise-decoupled NMR spectra were recorded with a Jeol FX-200 spectrometer at 50.1 MHz and a Bruker DPX-300 spectrometer at 75.5 MHz, using $CDCl_3$ (δ = 77 ppm), $(CD_3)_2CO$ (δ = 206 ppm) or TSP (δ = 0 ppm) as internal standards. Column chromatography was performed on Merck silica gel 60 (0.040−0.063 mm, 230−400 mesh) and spots were detected with UV light, $KMnO_4$ spraying, ninhydrin staining (0.2 % in ethanol) or by staining with 4,4′-methylenebis(*N*,*N*-dimethylaniline) and ninhydrin (TDM staining). Dry diethyl ether (ether, E) was obtained by distillation from P_2O_5. Dry petroleum ether of boiling range 40−60 °C (PE) and dry dichloromethane (DCM) were obtained by distillation from CaH_2. Dry tetrahydrofuran (THF) was obtained by drying with sodium/benzophenone. Methyl iodide (99 % ^{13}C) was purchased from Cambridge Isotope Laboratories, Inc. All other reagents were purchased from Aldrich Chemical Co. or Acros Chimica.

Enzyme Isolation: *Clostridium tetanomorphum* was obtained from the DSMZ micro-organism stock (Germany). For growth of the bacteria and harvesting of the enzyme, the original procedure by Barker[23] was applied. After purification with charcoal, no further purification was performed, but the protein mixture was stored at −20 °C until use. This seemed to have no detrimental effect on the activity of the enzyme.

[(Ethoxycarbonyl)methyl]triphenylphosphonium Bromide: Trifluoroacetic anhydride (14.1 mL, 100 mmol, 2 equiv.) was added to glacial acetic acid (2.40 mL, 42.3 mmol) in a dry 100-mL round-bottomed flask, equipped with a magnetic stirrer and cooled to 0 °C, using a dropping funnel. After stirring for 1 h, Br_2 (2.20 mL, 43 mmol) was added very slowly using a dropping funnel and stirring was continued overnight. The resulting pale-orange solution was cooled to 0 °C and distilled water (2.4 mL, 133 mmol) was slowly added using a dropping funnel. The solution was distilled using a mini-distillation setup with an oil-bath heated to 120 °C. After distillation of the bulk trifluoroacetic acid, the last traces were blown away with a soft nitrogen stream, giving an off-white crystalline solid (4.57 g). A further 0.13 g was obtained after concentration of the distillate using a steady nitrogen flow to give a total yield of 4.70 g (34 mmol) of bromoacetic acid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.9 (s, 2 H, 2-H), 9.7 (br. s, 1 H, 1-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 25.2 (C-2), 173.1 (C-1) ppm. $SOCl_2$ (42 mmol, 1.2 equiv.) was added at 0 °C to a 100-mL round-bottomed flask, charged with a solution of bromoacetic acid (35 mmol) in dry diethyl ether (5 mL) and equipped with a $CaCl_2$

tube, using a dropping funnel. The mixture was stirred at 0 °C for 1 h, then at room temperature for 2 h. Subsequently, ether and excess $SOCl_2$ were distilled off using a mini-distillation setup. Dry ethanol (10 mL, 5 equiv.) was added and the mixture was stirred overnight. The reaction mixture was taken up in ether (50 mL), extracted once with saturated sodium hydrogencarbonate solution (10 mL), once with water (10 mL) and then dried with anhydrous $MgSO_4$. After careful concentration, the yield was 5.5 g (95 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31 (t, $^3J_{H,H}$ = 7.2 Hz, 3 H, CH_3), 3.85 (s, 2 H, 1-H), 4.21 (q, $^3J_{H,H}$ = 7.2 Hz, 2 H, OCH_2) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.89 (CH_3), 25.86 (CH_2Br), 62.23 (CH_2), 167.14 (CO) ppm. A solution of ethyl bromoacetate (1.53 g, 10 mmol) in ethyl acetate (10 mL) was slowly added to a stirred solution of triphenylphosphane (2.75 g, 10.5 mmol, 1.05 equiv.) in ethyl acetate (50 mL), using a dropping funnel. The reaction mixture was stirred overnight and the white precipitate was filtered off, washed with ethyl acetate (10 mL) and dried in vacuo at 50 °C for 4 h; 4.05 g (9.7 mmol, 97 %) of the phosphonium salt was obtained. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.05 (t, $^3J_{H,H}$ = 7.1 Hz, 3 H, CH_3), 4.03 (q, $^3J_{H,H}$ = 7.1 Hz, 2 H, OCH_2), 5.51 (d, $^2J_{P,H}$ = 13.8 Hz, 2 H, 1-H), 7.93−7.61 (m, 15 H, 3*Ph) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 13.64 (CH_3), 33.1 (d, $^1J_{P,C}$ = 60.1 Hz, C-1), 62.8 (CH_2), 117.8 (d, $^1J_{P,C}$ = 89 Hz, C-1′), 130.1 (d, $^3J_{P,C}$ = 13 Hz, C-3′), 133.8 (d, $^2J_{P,C}$ = 10 Hz, C-2′), 135.1 (C-4′), 164.37 (C-2) ppm.

[1,2-Bis(ethoxycarbonyl)ethylidene]triphenylphosphorane (5): Phosphonium bromide (8.5 g, 20 mmol) was dissolved in DCM (50 mL) and extracted with NaOH (2 equiv.), dissolved in water (50 mL). The organic layer was collected and dried with $MgSO_4$, filtered and the solvent was evaporated. The resulting phosphorane **4** was redissolved in ethyl acetate (100 mL), solid K_2CO_3 (5.52 g, 2 equiv.) and ethyl bromoacetate (2.4 mL, 1.1 equiv.) were added and the mixture was refluxed for 4 h. The reaction mixture was subsequently filtered and the solids rinsed with ethyl acetate (25 mL). The filtrate was concentrated to give **5** (6.57 g, 75 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.11 (t, $^3J_{H,H}$ = 7.2 Hz, 6 H, CH_3), 2.95 (br. d, $^3J_{P,H}$ = 20 Hz, 2 H, 3-H), 3.95 (q, $^3J_{H,H}$ = 7.2 Hz, 4 H, OCH_2), 7.30−7.76 (m, 15 H, 3 × Ph) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.07 (CH_3), 57.3 (C-3), 59.72 (OCH_2), 128.23 (Ph), 128. 84 (Ph), 131.5 (Ph), 133.59 (Ph), 175.02 (CO) ppm.

Diethyl Itaconate (6): To a stirred solution of **5** (1.64 g, 3.78 mmol) in DCM (50 mL) was slowly added a 37 % aqueous formaldehyde solution (3.65 mL, 1.2 equiv.). The mixture was stirred at room temperature for 4 h and subsequently extracted with brine. The organic layer was dried with $MgSO_4$, filtered and the solvents were evaporated. The resulting raw product was purified by column chromatography (PE/E, 90:10). Yield 0.59 g (85 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26 (t, 3 H, CH_3), 1.30 (t, 3 H, CH_3), 3.35 (s, 2 H, 3-H), 4.16 (q, 2 H, OCH_2), 4.28 (q, 2 H, OCH_2), 5.78 (d, 1 H, 5-H) 6.37 (d, 1 H, 5-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.2 (2 × CH_3), 37.8 (C-3), 60.8 (OCH_2), 61.0 (OCH_2), 128.0 (C-5), 134.7 (C-2), 166.1 (CO), 170.6 (CO) ppm.

Mesaconic Acid (7): Diethyl itaconate (1.46 g, 7.89 mmol) was refluxed in toluene (20 mL) in the presence of DBU (1 equiv.) under exclusion of water, and the rearrangement of the double bond was monitored by TLC (PE/E, 90:10). When the reaction was complete, the solution was extracted with 1 M HCl, water, brine and the organic layer dried with $MgSO_4$. After evaporation of the solvent, concentrated HCl (10 mL) was added and the mixture was refluxed for 3 h and subsequently cooled to 0 °C using an ice bath. A white solid precipitated and was filtered through a glass filter after 15 min. The solid was rinsed carefully with cold concentrated hydrochloric acid (5 mL), after which the filter cake was air-dried by suction for 3 h. The yield of **7** was 0.79 g (6.1 mmol, 80 %). ^{1}H NMR [300 MHz, $(CD_3)_2CO$]: δ = 2.24 (d, $^4J_{H,H}$ = 1.7 Hz, 3 H, 5-H), 6.78 (q, $^4J_{H,H}$ = 1.7 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 13.28 (C-5), 133.57 (C-3), 143.39 (C-2), 165.88 (CO), 167.07 (CO) ppm.

[^{13}C]Mesaconic Acid (7a): Yield 81 %. ^{1}H NMR (300 MHz, $(CD_3)_2CO$]: δ = 2.24 (dd, $^1J_{C,H}$ = 129.41, $^4J_{H,H}$ = 1.7 Hz, 3 H, 5-H), 6.78 (dq, $^4J_{H,H}$ = 1.7, $^3J_{C,H}$ = 7.7 Hz, 1 H, 3-H) ppm. ^{13}C NMR [75 MHz, $(CD_3)_2CO$]: δ = 14.00 (C-5), 126.41 (C-3), 128.08 (d, $^1J_{C,C}$ = 23.33 Hz, C-2), 166.5, 172.84 ppm.

(2*S*,3*S*)-3-Methylaspartic Acid (10): 7 (5 g, 38.5 mmol) was dissolved in concentrated ammonia (15 mL). The solution was concentrated to dryness and the resulting ammonium salt of **7** was dissolved in 75 mL of a solution containing NH_4Cl (5.35 g per 100 mL), $MgCl_2{\cdot}6H_2O$ (4.06 g per 100 mL) and KCl (0.75 g per 100 mL) and the pH was adjusted to 9 using concentrated ammonia. The solution was transferred to a stoppered 250-mL Erlenmeyer flask and subsequently enzyme solution (250 μL) was added. The mixture was placed in a bath at 32 °C and shaken vigorously overnight. Then an aliquot was taken and ^{1}H NMR indicated 65 % conversion. The enzymes were denatured by acidifying to pH = 1 and heating to 80 °C for 10 min. The mixture was filtered and extracted with ether (2 × 50 mL). The organic layer was concentrated and **7** (1.5 g) was recovered. The water layer was concentrated to half the volume, ethanol (20 mL) was added and the pH adjusted to 3.1. Standing overnight at −20 °C gave clear crystals which were subsequently collected. After drying in vacuo, the yield was 3.4 g (60 %). Three cycles gave a total yield of 90 %. ^{1}H NMR (200 MHz, H_2O): δ = 1.18 (d, $^3J_{H,H}$ = 7.6 Hz, 3 H, 5-H), 2.95, (dq, $^3J_{H,H}$ = 7.6, $^3J_{H,H}$ = 3.2 Hz, 1 H, 3-H), 4.16 (dq, $^3J_{H,H}$ = 3.2, $^4J_{H,H}$ = 0.5 Hz, 1 H, 2-H) ppm. ^{13}C NMR (75.5 MHz, H_2O): δ = 12.93 (C-5), 39.48 (C-3), 54.62 (C-2), 169.9 (CO), 175.8 (CO) ppm.

[5-^{13}C]-(2*S*,3*S*)-3-Methylaspartic Acid (10a): Prepared as **10**. Unconverted [^{13}C]mesaconic acid could be recovered and reused: Total yield 92 %. ^{1}H NMR (300 MHz, H_2O, 300 K): δ = 1.30 (ddd, $^4J_{H,H}$ = 0.5, $^3J_{H,H}$ = 7.6, $^1J_{C,H}$ = 126.2 Hz, 3 H, 5-H), 3.10, (ddq, $^3J_{H,H}$ = 7.6, $^3J_{H,H}$ = 3.2, $^2J_{C,H}$ = 5.6 Hz, 1 H, 3-H), 4.16 (ddq, $^3J_{H,H}$ = 3.2, $^3J_{C,H}$ = 6.5, $^4J_{H,H}$ = 0.5 Hz, 1 H, 2-H) ppm. ^{13}C NMR (75.5 MHz, H_2O): δ = 12.20 (C-5) ppm.

1-Isopropyl (2*S*,3*S*)-3-Methyl-*N*-(trifluoroacetamido)aspartate: Trifluoroacetic anhydride (5 g, 24 mmol) was added dropwise over 10 min to dried **10** (0.35 g) in a flame-dried 25-mL round-bottomed flask, equipped with a pressure-equalizing dropping funnel equipped with a calcium chloride drying tube, and cooled to 0 °C. The reaction mixture was stirred for 2 h while the temperature was allowed to reach room temperature. Subsequently, the trifluoroacetic anhydride and the formed trifluoroacetic acid were removed using an oil pump. Dried 2-propanol (1.85 mL, 10 equiv.) was added to the cyclic anhydride formed and the mixture was stirred at room temp. for 2 h. Excess 2-propanol was removed using an oil pump. The crude product was taken up in ether (25 mL) and washed with $NaHCO_3$ (5 mL), H_2O (5 mL), brine, dried with $MgSO_4$ and subsequently filtered and the solvents were evaporated to dryness. Yield 0.66 g (2.34 mmol, 99 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (d, $^3J_{H,H}$ = 5.9 Hz, 3 H, CH_3), 1.29 (d, $^3J_{H,H}$ = 5.9 Hz, 3 H, CH_3), 1.36 (d, $^3J_{H,H}$ = 7.3 Hz, 3 H, 5-H), 3.08 (dq, $^3J_{H,H}$ = 7.3, $^3J_{H,H}$ = 4.0 Hz, 1 H, 3-H), 4.80 (dd, $^3J_{H,H}$ = 4.0, $^3J_{H,H}$ = 8.0 Hz, 1 H, 2-H), 5.11 (dq, $^3J_{H,H}$ = 5.9, $^3J_{H,H}$ = 5.9 Hz, 1 H, OCH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 13.17 (C-5), 21.49 (CH_3), 21.56 (CH_3), 41.68 (C-3), 54.23 (C-2), 71.0 (OCH), 118.75 (CF_3), 159.10 (CO), 168.26 (C-4), 177.68 (C-1) ppm.

1-Isopropyl [4-^{13}C]-(2*S*,3*S*)-3-Methyl-*N*-(trifluoroacetamido)aspartate: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (d, $^3J_{H,H}$ = 5.9 Hz, 3 H, CH_3), 1.29 (d, $^3J_{H,H}$ = 5.9 Hz, 3 H, CH_3), 1.36 (dd, $^1J_{C,H}$ = 129.41, $^3J_{H,H}$ = 7.3 Hz, 3 H, 5-H), 3.08 (m, 1 H, 3-H), 4.80 (dd, $^3J_{H,H}$ = 4.0, $^3J_{H,H}$ = 8.0 Hz, 1 H, 2-H), 5.11 (dq, $^3J_{H,H}$ = 5.9, $^3J_{H,H}$ = 5.9 Hz, 1 H, OCH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 13.17 (C-5), 21.49 (CH_3), 21.56 (CH_3), 41.68 (d, C-3), 54.23 (C-2), 71.0 (OCH), 118.75 (CF_3), 159.10 (CO), 177.68 (CO) ppm.

1-Isopropyl (2*S*,3*S*)-3-Methyl-*N*-(trifluoroacetamido)homoserinate: A solution of *N*-methylmorpholine (0.31 mL, 2.81 mmol) was added dropwise to a three-necked round-bottomed flask charged with a solution of the protected aspartate ester (0.73 g, 2.56 mmol) in dry THF (50 mL), equipped with a pressure-equalizing dropping funnel, under dry argon and cooled to −50 °C. Subsequently, isobutyl chloroformate (0.35 mL, 2.7 mmol) was added slowly using a syringe and the mixture was stirred for 5 min. The resulting suspension was quickly filtered into a solution of sodium borohydride (77 mg, 2.1 mmol) in dry THF at −20 °C and was stirred for 3 h. The reaction was quenched by addition of a 1:1 acetic acid/water mixture (1 mL). The solvents were evaporated and the resulting oil was taken up in ether (50 mL), extracted with water and brine and dried with $MgSO_4$. The product was purified using column chromatography (PE/E, 70:30) with acetic acid (5 drops) added to the eluent. Yield 400 mg (58 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.77 (d, $^3J_{H,H}$ = 7.1 Hz, 3 H, 5-H), 1.29 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, CH_3), 1.31 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, CH_3), 2.44 (m, 1 H, 3-H), 3.24 (dd, $^3J_{H,H}$ = 12.1, $^3J_{H,H}$ = 10.6 Hz, 1 H, 4-H), 3.57 (dd, $^3J_{H,H}$ = 12.1, $^3J_{H,H}$ = 4.9 Hz, 1 H, 4-H), 4.84 (dd, $^3J_{H,H}$ = 7.8, $^3J_{H,H}$ = 2.8 Hz 1 H, 1-H), 5.13 (h, $^3J_{H,H}$ = 6.2 Hz, 1 H, CH), 7.28 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ= 10.44 (C-5), 21.69 (C-7/C-8), 38.74 (C-3), 53.50 (C-2), 63.91 (C-4), 70.57 (C-6), 115.61 (d, $^1J_{C,F}$ = 287.3 Hz, C-9), 158.10 (d, $^2J_{C,F}$ = 38.0 Hz, C-10), 170.06 (C-1) ppm.

1-Isopropyl [3-^{13}C]-(2*S*,3*S*)-3-Methyl-*N*-(trifluoroacetamido)homoserinate: The reaction was performed as for unlabelled homoserine. The yield was 65 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.77 (dd, $^3J_{H,H}$ = 7.1, $^1J_{C,H}$ = 126.7 Hz, 3 H, 5-H), 1.29 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, CH_3), 1.31 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, CH_3), 2.44 (m, 1 H, 3-H), 3.24 (ddd, $^3J_{H,H}$ = 12.1, $^3J_{H,H}$ = 10.6, $^3J_{C,H}$ = 1.7 Hz, 1 H, 4-H), 3.57 (ddd, $^3J_{H,H}$ = 12.1, $^3J_{H,H}$ = 4.9, $^3J_{C,H}$ = 1.6 Hz, 1 H, 4-H), 4.84 (ddd, $^3J_{H,H}$ = 7.8, $^3J_{H,H}$ = 2.8, $^3J_{C,H}$ = 2.9 Hz 1 H, 1-H), 5.13 (H, $^3J_{H,H}$ = 6.2 Hz, 1 H, CH), 7.28 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 10.68 (C-5), 21.51 (C-7/C-8), 38.36 (d, $^1J_{C,C}$ 35.15 Hz, C-3), 53.84 (C-2), 63.83 (C-4), 70.43 (C-6), 115.61 (d, $^1J_{C,F}$ = 287.3 Hz, C-9), 158.10 (d, $^2J_{C,F}$ = 38.0 Hz, C-10), 170.08 (C-1) ppm.

Isopropyl 4-Iodo-3-methyl-2-(trifluoroacetamido)butanoate (12): A solution of iodine (0.99 g, 1.99 equiv.) in dry DCM (10 mL) was added dropwise to a solution of triphenylphosphane (1.02 g, 2 equiv.) in dry DCM. A mixture of imidazole (0.3 g, 2.2 equiv.) and protected homoserine (0.5 g, 1.9 mmol), dissolved in dry DCM (10 mL), was added dropwise to the resulting pale-coloured liquid. After 2 h, TLC indicated complete conversion. After filtering off the solids, the product was extracted with 1 M HCl solution, water and brine and dried with $MgSO_4$. The resulting oil was purified using column chromatography (PE/E, 85:15) with glacial acetic acid (5 drops) added to the eluent. Yield 0.70 g (95 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.05 (d, $^3J_{H,H}$ = 6.9 Hz, 3 H, 5-H), 1.30 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 1.31 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 2.43 (m, 1 H, 3-H), 2.96 (dd, $^3J_{H,H}$ = 8.3, $^3J_{H,H}$ = 10.2 Hz, 1 H, 4-H), 3.20 (dd, $^3J_{H,H}$ = 5.5, $^3J_{H,H}$ = 10.2 Hz, 1 H, 4-H), 4.83 (dd, $^3J_{H,H}$ = 3.8, $^3J_{H,H}$ = 8.6 Hz, 1 H, 2-H), 5.11 (sept, $^3J_{H,H}$ = 6.3 Hz, 1 H, CH), 6.95 (d, $^3J_{H,H}$ = 8.6 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 8.2 (C-4), 15.32 (C-5), 21.65 (2 × CH_3), 39.47 (C-3), 55.78 (C-2), 70.83 (OCH), 115.51 (q, $^1J_{C,F}$ = 287.6 Hz, CF_3), 157.3 (q, $^2J_{C,F}$ = 37.7 Hz, CO), 169.08 (C-1) ppm.

Isopropyl [^{13}C]-4-Iodo-3-methyl-2-(trifluoroacetylamino)butanoate (12a): The same procedure was applied as for **11**. The yield was 92 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.05 (dd, $^1J_{C,H}$ = 127.35, $^3J_{H,H}$ = 6.9 Hz, 3 H, 5-H), 1.30 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 1.31 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 2.43 (m, 1 H, 3-H), 2.96 (ddd, $^3J_{H,H}$ = 8.3, $^3J_{H,H}$ = 10.2, $^3J_{C,H}$ = 3.7 Hz, 1 H, 4-H), 3.20 (ddd, $^3J_{H,H}$ = 5.5, $^3J_{H,H}$ = 10.2, $^3J_{C,H}$ = 5.3 Hz, 1 H, 4-H), 4.83 (ddd, $^3J_{H,H}$ = 3.8, $^3J_{H,H}$ = 8.6, $^3J_{C,H}$ = 4.7 Hz, 1 H, 2-H), 5.11 (sept, $^3J_{H,H}$ = 6.3 Hz, 1 H, CH), 6.95 (d, $^3J_{H,H}$ = 8.6 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ= 8.2 (C-4), 15.32 (C-5), 21.65 (2 × CH_3), 39.47 (d, $^1J_{C,C}$ = 35.3 Hz, C-3), 55.78 (C-2), 70.83 (OCH), 115.51 (q, $^1J_{C,F}$ = 287.6 Hz, CF_3), 157.3 (q, $^2J_{C,F}$ = 37.7 Hz, CO), 169.08 (C-1) ppm.

(2*S*)-Valine·HCl (1): 11 (1.5 g, 3.9 mmol) was dissolved in methanol, 5 % Pd/C (1.5 g) was added and hydrogen gas was bubbled through. After TLC indicated complete disappearance of the starting material, the reaction mixture was flushed with argon and filtered through Celite under argon. Subsequently, the solution was concentrated to dryness and the protected valine was redissolved in 2-propanol (20 mL). 2 M KOH solution (10 mL) was added and the mixture was stirred for 4 h. The solvent was evaporated and the residue was redissolved in water. Dowex H^+ was added and the mixture was stirred gently for 1 h. The resin was rinsed with water (100 mL) and eluted with 0.2 M NH_3. The fractions giving a ninhydrin stain were collected and the solvents evaporated. 1 M HCl (10 mL) was added and the solution lyophilized giving a yield of 0.46 g of valine·HCl (77 %). ^{1}H NMR (300 MHz, D_2O): δ = 1.02 (d, $^3J_{H,H}$ = 7.2 Hz, 4-H), 1.04 (d, $^3J_{H,H}$ = 7.1 Hz, 5-H), 2.35 (dqq, $^3J_{H,H}$ = 7.2, $^3J_{H,H}$ = 7.1, $^3J_{H,H}$ = 4.4 Hz, 1 H, 3-H), 3.94 (d, $^3J_{H,H}$ = 4.4 Hz, 1 H, 2-H) ppm. ^{13}C NMR (75.5 MHz, D_2O): δ= 17.47 (C-4), 17.96 (C-5), 32.58 (C-3), 62.61 (C-2), 183.83 (C-1) ppm.

(2*S*,3*S*)-Valine·HCl (1a): The procedure as described for the preparation of **1** was repeated with **11a** (0.8 g, 2.1 mmol), giving **1a** in a yield of 81 % (0.26 g, 1.7 mmol). ^{1}H NMR (300 MHz, D_2O): δ = 1.02 (dd, $^1J_{C,H}$ = 125.1, $^3J_{H,H}$ = 7.2 Hz, 3 H, 4-H), 1.04 (dd, $^3J_{H,H}$ = 7.1, $^3J_{C,H}$ = 5.4 Hz, 3 H, 5-H), 2.35 (ddqq, $^3J_{H,H}$ = 7.2, $^3J_{H,H}$ = 7.1, $^3J_{H,H}$ = 4.4, $^2J_{C,H}$ = 4.4 Hz, 1 H, 3-H), 3.94 (dd, $^3J_{C,H}$ = 4.9, $^3J_{H,H}$ = 4.4 Hz, 1 H, 2-H) ppm. ^{13}C NMR (75.5 MHz, D_2O): δ= 17.47 (C-4), 17.96 (C-5), 32.58 (d, $^1J_{C,C}$ = 35.5 Hz, C-3), 62.61 (C-2), 183.83 (C-1) ppm.

Diethyl Ethylidenesuccinate (8): Acetaldehyde (0.73 mL, 13.0 mmol) in DCM (5 mL) was slowly added to a stirred solution of **5** (5 g, 11.7 mmol) in DCM (50 mL). The mixture was stirred at room temp. for 4 h and subsequently extracted with brine. The organic layer was dried with $MgSO_4$ and the solvents were evaporated. The resulting raw product was purified by column chromatography (PE/E). Yield: 90 % (2.1 g). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.25 (t, $^3J_{H,H}$ = 7 Hz, 3 H, CH_3), 1.28 (t, $^3J_{H,H}$ = 7 Hz, 3 H, CH_3), 1.84 (d, 3 H, 6-H), 3.35 (s, 2 H, 3-H) 4.15 (q, $^3J_{H,H}$ = 7 Hz, 2 H, OCH_2), 4.20 (q, $^3J_{H,H}$ = 7 Hz, 2 H, OCH_2), 6.13 (q, 1 H, 5-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.1 (C-6), 14.5 (2 × CH_3), 32.1 (C-3), 60.7 (2 × CH_2), 126.9 (C-5), 140.1 (C-2), 166.8 (CO), 170.7 (CO) ppm.

2-Ethylfumaric Acid (9): 8 (1.5 g, 7.5 mmol) was refluxed in toluene (20 mL) in the presence of DBU (1 equiv.) and the rearrangement of the double bond was monitored by TLC (PE/E, 90:10). When

only traces of **8** remained, the solution was extracted with 1 M HCl, water, brine and the organic layer was dried with $MgSO_4$. After evaporation of the solvent, concentrated HCl (10 mL) was added and the mixture was refluxed for 3 h and subsequently cooled to 0 °C using an ice bath. A white solid precipitated and was filtered through a glass filter after 15 min. The solid was rinsed carefully with cold concentrated hydrochloric acid (5 mL), after which the filter cake was air-dried by suction for 3 h. The yield of 2-ethylfumaric acid was 0.87 g (6.1 mmol, 81 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.08 (t, $^3J_{H,H}$ = 7.4 Hz, 3 H, 6-H), 2.78 (q, $^3J_{H,H}$ = 7.4 Hz, 2 H, 5-H), 6.75 (s, 1 H, 3-H) ppm.

3-Ethylaspartic Acid (13): 9 (5 g, 35 mmol) was dissolved in concentrated ammonia (15 mL). The solution was concentrated to dryness and the resulting ammonium salt was dissolved in 75 mL of a solution containing NH_4Cl (5.35 g per 100 mL), $MgCl_2{\cdot}6H_2O$ (4.06 g per 100 mL) and KCl (0.75 g per 100 mL) and the pH was adjusted to 9 using concentrated ammonia. The solution was transferred to a stoppered 250-mL Erlenmeyer flask and enzyme solution (250 μL) was added. The mixture was shaken vigorously overnight in a bath at 32 °C. After one night an aliquot was taken and ^{1}H NMR indicated 60 % conversion. The enzymes were denatured by acidifying to pH = 1 and heating to 80 °C for 10 min. The mixture was filtered and extracted with ether (2 × 50 mL). The organic layer was concentrated and **9** (1.3 g) was recovered. The water layer was concentrated to half volume, ethanol (20 mL) was added and the pH adjusted to 3.1. Standing overnight at −20 °C gave clear crystals which were subsequently collected. After drying in vacuo, the yield was 3.38 g (60 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 3 H, 5-H), 1.7 (m, 2 H, 4-H), 2.90 (quint, $^3J_{H,H}$ = 4.2 Hz, 1 H, 3-H), 4.06 (d, $^3J_{H,H}$ = 4.2 Hz, 1 H, 2-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 12.23 (C-5), 21.46 (C-4), 48.07 (C-3), 55.86 (C-2), 172.96 (C-1), 178.09 (C-6) ppm.

1-Isopropyl (2*S*,3*S*)-3-Ethyl-*N*-(trifluoroacetamido)aspartate: Trifluoroacetic anhydride (6 g, 29 mmol) was added dropwise over 10 min to dried **13** (0.5 g, 3.1 mmol) in a flame-dried 25-mL round-bottomed flask equipped with a pressure-equalizing dropping funnel fitted with a calcium chloride drying tube and cooled to 0 °C. The reaction mixture was stirred for 2 h while the temperature was allowed to reach 20 °C. Subsequently, the TFA anhydride and the formed TFA were removed using an oil pump equipped with a cold trap. To the cyclic anhydride formed dried 2-propanol (10 equiv.) was added and the mixture was stirred for 2 h at room temp. Excess 2-propanol was removed using an oil pump. The crude product was taken up in ether (25 mL) and washed with $NaHCO_3$ (5 mL), H_2O (5 mL), brine, dried with $MgSO_4$ and subsequently filtered and the solvents were evaporated to dryness. Yield 99 % (0.92 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.95 (t, $^3J_{H,H}$ = 7.51 Hz, 3 H, 5-H), 1.20 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 1.22 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 1.71 (p, 7.5 Hz, 2 H, 4-H), 2.93 (ddd, $^3J_{H,H}$ = 6.3, $^3J_{H,H}$ = 7.1, $^3J_{H,H}$ = 8.7 Hz, 1 H, 3-H), 4.79 (dd, $^3J_{H,H}$ = 8.7 Hz, 1 H, 2-H), 4.95 (H, $^3J_{H,H}$ = 6.3 Hz, 1 H, OCH), 8.6 (d, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 10.34 (CH_3), 20.68 (2 × CH_3), 21.38 (CH_2), 40.98 (CH), 53.16 (NCH), 69.19 (OCH), 115.86 (q, $^1J_{C,F}$ = 287 Hz, CF_3), 157.4 (q, $^2J_{C,F}$ = 42 Hz, CF_3), 168.29 (CO), 173.07 (CO) ppm.

Isopropyl (2*S*,3*S*)-3-Ethyl-*N*-(trifluoroacetamido)homoserinate: A solution of *N*-methylmorpholine (0.29 mL, 2.6 mmol) in THF (5 mL) was added dropwise under dry argon to a three-necked round-bottomed flask charged with a solution of the previously obtained protected ethyl aspartate ester (0.70 g, 2.4 mmol) in dry THF (50 mL), equipped with a pressure-equalizing dropping funnel, and cooled to −50 °C. Subsequently, isobutyl chloroformate (0.32 mL) was added slowly using a syringe and the mixture was stirred for 5 min. The resulting suspension was quickly filtered into a solution of $NaBH_4$ (75 mg) in dry THF at −20 °C and was stirred for 3 h. The reaction was quenched by addition of a 1:1 acetic acid/water mixture (1 mL). The solvents were evaporated and the resulting oil was taken up in ether (50 mL), extracted with water and brine and dried with $MgSO_4$. The product was purified using column chromatography (PE/E, 70:30) with acetic acid (5 drops) added to the eluent. The yield was 450 mg (65 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.96 (t, $^3J_{H,H}$ = 7.4 Hz, 3 H, 5-H), 1.24 (m, 2 H, 4-H), 1.28 (d, $^3J_{H,H}$ = 6.1 Hz, 3 H, CH_3), 1.30 (d, $^3J_{H,H}$ = 6.1 Hz, 3 H, CH_3), 2.18 (m, 1 H, 3-H), 3.55 (dd, $^2J_{H,H}$ = 11.7, $^3J_{H,H}$ = 10.3 Hz, 1 H, 6-H), 3.76 (dd, $^2J_{H,H}$ = 11.7, $^3J_{H,H}$ = 4.4 Hz, 1 H, 6-H), 4.82 (dd, $^3J_{H,H}$ = 3.0, $^3J_{H,H}$ = 7.9 Hz, 1 H, 2-H), 4.99 (q, $^3J_{H,H}$ = 6.2 Hz, 1 H, CH), 7.69 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 11.83 (C-5), 19.68 (C-4), 21.51 (CH_3), 21.57 (CH_3), 45.06 (C-3), 53.74 (C-2), 62.29 (C-6), 70.33 (OCH), 115.64 (q, $^1J_{C,F}$ = 287.3 Hz, CF_3), 157.82 (q, $^2J_{C,F}$ = 37.8 Hz, CO), 170.12 (C-1) ppm.

Isopropyl (2*S*,3*S*)-3-Ethyl-4-iodo-2-(trifluoroacetylamino)butyrate (15): A solution of iodine (0.80 g, 1.99 equiv.) in dry DCM (10 mL) was added dropwise to a solution of triphenylphosphane (0.82 g, 2 equiv.) in dry DCM (10 mL). A mixture of imidazole (0.3 g) and the previously obtained alcohol (0.45 g, 1.6 mmol), dissolved in dry DCM (10 mL), was added dropwise to the resulting pale-coloured liquid. After 2 h, TLC indicated complete conversion. After filtering off the solids, the product was extracted with 1 M HCl solution, water and brine and dried with $MgSO_4$. The resulting oil was purified using column chromatography (PE/E, 85:15) with glacial acetic acid (5 drops) added to the eluent. The yield was 0.59 g (95 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.98 (t, $^3J_{H,H}$ = 7.3 Hz, 3 H, 5-H), 1.29 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 1.31 (d, $^3J_{H,H}$ = 6.3 Hz, 3 H, CH_3), 1.49 (m, 2 H, 4-H), 2.08 (m, 1 H, 3-H), 3.19 (m, 1 H, 6-H), 4.82 (dd, $^3J_{H,H}$ = 4.16, $^3J_{H,H}$ = 8.3 Hz, 1 H, 2-H), 5.11 (sept, $^3J_{H,H}$ = 6.3 Hz, 1 H, CH), 7.00 (d, $^3J_{H,H}$ = 8.6 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ= 5.89 (C-6), 11.34 (C-5), 21.59 (2 × CH_3), 23.66 (C-4), 45.06.47 (C-3), 55.31 (C-2), 70.66 (OCH), 115.66 (q, $^1J_{C,F}$ = 287.6 Hz, CF_3), 157.15 (q, $^2J_{C,F}$ = 38.2 Hz, CO), 169.36 (C-1) ppm.

(2*S*,3*S*)-Isoleucine·HCl (2): 15 (150 mg, 0.4 mmol) was dissolved in dry methanol (25 mL) in a three-necked round-bottomed flask and triethylamine (2 equiv.) and 5 % Pd/C (0.75 g) were added. Hydrogen was bubbled through the solution for 3 h, after which the solution was purged with argon. The solution was filtered through Celite and purified by chromatography through a short column (PE/E, 50:50). Subsequently, a portion of the protected isoleucine (81 mg, 82 %) was dissolved in 2-propanol (10 mL), 2 M KOH (5 mL) solution was added and the mixture was stirred for 6 h. The solvent was evaporated and the residue was redissolved in water. Dowex H^+ was added and the mixture stirred gently for 30 min. The resin was rinsed with water (100 mL) and eluted with 0.2 M NH_3. The fractions giving a ninhydrin stain were collected and the solvents evaporated. 1 M HCl (2 mL) was added and the solution lyophilized yielding isoleucine·HCl (37 mg, 73 %). ^{1}H NMR (300 MHz, D_2O): δ = 0.85 (dd, $^3J_{H,H}$ = 7.35, $^3J_{H,H}$ = 7.44 Hz, 3 H, 5-H), 0.94 (d, $^3J_{H,H}$ = 7.0 Hz, 3 H, 6-H), 1.24 (m, 1 H, 4-H), 1.42, (m, 1 H, 4-H), 1.98 (m, 1 H, 3-H), 3.97 (d, $^3J_{H,H}$ = 3.9 Hz, 1 H, 2-H) ppm. ^{13}C NMR (75.5 MHz, D_2O): δ= 11.70 (C-5), 14.92 (C-6), 25.90 (C-4), 36.53 (C-3), 57.97 (C-2), 182.68 (C-1) ppm.

Ethyl Pyruvate (16): 2 M NaOH solution (50 mL) was added to a solution of **4** (4.16 g, 9.7 mmol) in dichloromethane (75 mL) in a separating funnel. After vigorous shaking, the two layers were sepa-

rated and the dichloromethane layer was collected. The water layer was extracted with dichloromethane (2 × 25 mL) and the combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. The resulting ylide was redissolved in dichloromethane (90 mL) and transferred to a 100-mL flask equipped with a stirring bar. Methyl iodide (0.77 mL, 12.6 mmol, 1.3 equiv.) was added using a syringe. After stirring overnight, the mixture of non-, mono- and dimethylated product was concentrated in vacuo, giving a yield of 4.48 g. Yield of the monomethylated product was 85 % (8.0 mmol) according to ^{1}H NMR spectroscopy. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.05 (t, $^3J_{H,H}$ = 7.1 Hz, 3 H, CH_3), 1.69 (dd, $^3J_{H,H}$ = 7.21, $^3J_{P,H}$ = 18.5 Hz, 3 H, 3-H), 4.03 (q, $^3J_{H,H}$ = 7.1 Hz, 2 H, CH_2O), 6.21 (dq, $^2J_{P,H}$ = 14.2, $^3J_{H,H}$ = 7.21 Hz, 2 H, 1-H), 7.68−7.98 (m, 15 H, 3 × Ph) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 12.0 (CH_3), 13.2 (s, CH_3), 33.1 (d, $^1J_{P,C}$ = 60.1 Hz, C-1), 61.7 (OCH_2), 117.8 (d, $^1J_{P,C}$ = 89 Hz, C-1′), 130.1 (d, $^3J_{P,C}$ = 13 Hz C-3′), 133.8 (d, $^2J_{P,C}$ = 10 Hz, C-2′), 135.1 (C-4′) 167.4 (C-2) ppm. A 250-mL three-necked round-bottomed flask, equipped with stirring bar, non-pressure-equalizing dropping funnel and a gas outlet connected to a bubble counter filled with a solution of acetic acid and potassium iodide in water was flame-dried under nitrogen. The flask was charged with a solution of ylide (5 g, 12.8 mmol) in DCM (150 mL) and cooled to −60 °C, using an acetone/dry ice bath, while the dropping funnel was charged with a solution of dimethyl sulfide (2 equiv.) in dichloromethane (25 mL). Ozone was bubbled through the ylide solution in a steady stream until the solution turned blue. The excess ozone was purged from the solution using a nitrogen stream after which the dimethyl sulfide solution was added dropwise. The solution was warmed to 0 °C, after which PE (50 mL) was added, resulting in a turbid solution. The solution was passed through a glass filter filled with silica, which was rinsed with PE/E (50:50). The solution was then concentrated in vacuo to 10 mL and purified by chromatography through a short column (PE/E, 90:10), giving ethyl pyruvate (0.95 g, 65 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.50 (t, $^3J_{H,H}$ = 7.21 Hz, 3 H, CH_3), 2.44 (s, 3 H, 3-H), 4.30 (q, $^3J_{H,H}$ = 7.21 Hz, 2 H, OCH_2) ppm.

Ethyl 2-Oxobutyrate (17): Ethyl pyruvate (**16**) (2.22 mL, 20 mmol) was dissolved in ether (50 mL) and a solution of *N*-methyl-*N*-phenylhydrazine (2.31 mL, 0.98 equiv.) in ether (10 mL) was slowly added. After one night, TLC showed almost complete conversion. The yellow solution was concentrated in vacuo and the product purified by column chromatography (PE/E, 70:30), giving a 95 % yield of the hydrazone (4.25 g). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.42 (t, $^3J_{H,H}$ = 7.2 Hz, 3 H, CH_3), 2.05 (s, H3, 3-H), 3.42 (s, 3 H, NCH_3), 4.34 (q, $^3J_{H,H}$ = 7.2 Hz, 2 H, OCH_2), 6.96−7.34 (m, 5 H, Ph) ppm. The hydrazone (2.2 g, 10 mmol) was dissolved in dry THF (10 mL) and added dropwise to a solution of LDA (1.1 equiv.) in dry THF (50 mL) at −80 °C [prepared by addition of 1.6 M BuLi (7 mL) to a solution of diisopropylamine (1.54 mL) in dry THF]. The reaction was quenched by addition of MeI (0.93 mL, 1.5 equiv.) and stirred for 4 h. The reaction mixture was extracted with water and brine and the collected organic layers were dried with $MgSO_4$. Purification was performed by column chromatography (PE/E, 70:30) to give a yield of 75 % of the ethyl hydrazone; 1.2 equiv. of 1 M HCl was added to a solution of the hydrazone in THF (30 mL) and stirred for 2 h followed by addition of 1 M HCl (3 mL) and stirring for 10 min. After extraction with ether and careful evaporation of the solvents in vacuo, an 80 % yield of ethyl oxobutyrate was obtained (1.48 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.13 (t, $^3J_{H,H}$ = 7.2 Hz, 3 H, 4-H), 1.37 (t, $^3J_{H,H}$ = 7.2 Hz, 3 H, CH_3), 2.87 (q, $^3J_{H,H}$ = 7.2 Hz, 2 H, 3-H), 4.32 (q, $^3J_{H,H}$ = 7.2 Hz, 2 H, OCH_2) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 6.49 (C-4), 13.90 (CH_3), 32.69 (C-3), 62.24 (OCH_2), 161.09 (C-1), 195.04 (C-2) ppm.

***tert*-Butyl Bromoacetate (24):** Trifluoroacetic anhydride (14.1 mL, 100 mmol, 2 equiv.) was carefully added using a dropping funnel to glacial acetic acid (2.40 mL, 42.3 mmol) in a dry 100-mL round-bottomed flask equipped with a magnetic stirrer and cooled to 0 °C. After stirring for 1 h, Br_2 (2.20 mL, 43 mmol) was added very slowly using a dropping funnel and stirring was continued overnight. The resulting pale-orange solution was cooled to 0 °C and distilled water (2.4 mL, 133 mmol) was slowly added using a dropping funnel. The solution was distilled using a mini-distillation setup with an oil bath heated to 120 °C. After distilling off the bulk trifluoroacetic acid, the last traces were blown away with a soft nitrogen stream, giving an off-white crystalline solid (4.57 g). A further 0.13 g was obtained after concentration of the distillate using a steady nitrogen flow to give a total yield of 4.70 g (34 mmol) of bromoacetic acid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.9 (s, 2 H, 2-H), 9.7 (br. s, 1 H, 1-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 25.2 (C-2), 173.1 (C-1) ppm. A suspension of bromoacetic acid (1.18 g, 8.5 mmol), scandium triflate (2.55 g, 0.6 equiv.), *tert*-butyl alcohol (20 mL) and DMAP (5.2 g) in dry DCM (5 mL) was cooled to −5 °C in an ice/salt bath for 40 min. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (3.23 g) was added and the mixture stirred at −5 °C for 30 min and then allowed to come to room temp. in 2 h. The mixture was filtered, washed with 0.1 M HCl, 0.1 M Na_2CO_3 and water. The organic layer was dried with $MgSO_4$ and carefully concentrated to dryness, giving **24** (2.33 g, 35 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.48 (s, 9 H), 3.75 (s, 2 H, 2-H) ppm.

***tert*-Butyl Glycinate (25):** In a flask cooled to −40 °C, ammonia (100 mL) was condensed, and then diluted with anhydrous ether (100 mL). To this solution, *tert*-butyl bromoacetate (50 g, 0.25 mol) in diethyl ether (50 mL) was slowly added at −40 °C and the temperature was maintained for 2 h, then warmed to room temp. After stirring overnight, the ammonium bromide was filtered off and the solvent evaporated under reduced pressure, giving **25** in 87 % yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.47 (s, 9 H) 3.31 (s, 2 H, 2-H) ppm.

***tert*-Butyl *N*-(Diphenylmethylidene)glycinate (26):** The glycine ester **25** (10.5 mmol) was added to an equimolar amount of benzophenone imine in CH_2Cl_2 (20 mL per g of benzophenone imine) and was stirred at room temperature for 24 h with exclusion of moisture ($CaCl_2$ tube). The reaction mixture was filtered to remove NH_4Cl and the solvents were evaporated to dryness in a rotary evaporator. The residue was taken up in diethyl ether (equal in volume to the CH_2Cl_2), filtered, washed with water (equal volume to the ether), and dried with $MgSO_4$. Filtration and solvent removal were followed by recrystallization (ethanol/PE) resulting in a yield of 32.11 g (98 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.44 (s, 9 H), 4.12 (s, 2 H, 2-H), 7.4 (m, 10 H, 2 × Ph) ppm.

(4*S*)-4-Benzyl-3-(2-methylpropionyl)-2-oxazolidinone (20): A solution of (4*S*)-4-benzyl-3-propanoyloxazolidin-2-one (700 mg, 3.0 mmol) in THF (6 mL) was added dropwise to a stirred solution of sodium hexamethyldisilazide (1.0 M in THF, 3.3 mL, 3.3 mmol) in THF (15 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1 h, then iodomethane (0.37 mL, 6.0 mmol) was added dropwise. The reaction mixture was stirred at −78 °C for 4 h, then satd. ammonium chloride solution (15 mL) and water (6 mL) were added and the aqueous phase acidified to pH = 2 with sulfuric acid. The product was extracted with ethyl acetate (3 × 60 mL). The combined extracts were washed successively with satd. sodium

hydrogencarbonate solution (15 mL), sodium thiosulfate solution (15 mL) and brine (15 mL), dried with magnesium sulfate, filtered and concentrated in vacuo to give an oil. The product was purified by column chromatography (2−10 % ethyl acetate in PE) to afford (4*S*)-benzyl-3-(2-methylpropionyl)oxazolidin-2-one as a pale-yellow oil (536 mg, 72 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 [d, $^3J_{H,H}$ = 6.8 Hz, 3 H, 9-H (*R*)], 1.23 [d, $^3J_{H,H}$ = 6.7 Hz, 3 H, 9-H (*S*)], 2.77 (dd, $^2J_{H,H}$ = 13.4, $^3J_{H,H}$ = 9.5 Hz, 1 H, 6-H), 3.24 (dd, $^2J_{H,H}$ = 13.4, $^3J_{H,H}$ = 3.3 Hz, 1 H, 6-H), 3.75 (qq, $^3J_{H,H}$ = 6.7, $^3J_{H,H}$ = 6.8 Hz, 1 H, 8-H), 4.15 (dd, $^2J_{H,H}$ = 9.0, $^3J_{H,H}$ = 3.5 Hz, 1 H, 5-H), 4.19 (dd, $^2J_{H,H}$ = 9.0, $^3J_{H,H}$ = 7.4 Hz, 1 H, 5-H), 4.67 (m, 1 H, 4-H), 7.27 (m, 5 H, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.6 [C-9 (*R*)], 19.1 [C-9 (*S*)], 32.5 (C-8), 37.8 (C-6), 55.2 (C-4), 65.9 (C-5), 127−153 (Ar), 177.5 (C-7) ppm.

[2-^{13}C]-(4*S*)-4-Benzyl-3-(2-methylpropionyl)-2-oxazolidinone (20a): The above procedure was successfully reproduced starting with $^{13}CH_3I$ (4.5 g, 31.5 mmol), (4*S*)-4-benzyl-3-propanoyloxazolidin-2-one (5.3 g, 22.5 mmol), sodium hexamethyldisilazide (1.0 M in THF, 25 mL, 25 mmol) and THF (80 mL) giving a 1:8.4 mixture of (4*S*)-4-benzyl-3-[(2*R*)-2-methylpropionyl]oxazolidin-2-one and (4*S*)-4-benzyl-3-[(2*S*)-2-methylpropionyl]oxazolidin-2-one as products (4.0 g, 72 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 [dd, $^3J_{H,H}$ = 6.8, $^1J_{C,H}$ = 128.3 Hz, 0.2 H, 9-H (*R*)], 1.19 [dd, $^3J_{H,H}$ = 6.8, $^3J_{C,H}$ = 5.1 Hz, 2.8 H, 9-H (*R*)], 1.23 [dd, $^3J_{H,H}$ = 6.7, $^1J_{C,H}$ = 128.3 H, 2.8 Hz, 9-H (*S*)], 1.23 [dd, $^3J_{H,H}$ = 6.7, $^3J_{C,H}$ = 10.3 Hz, 0.2 H, 9-H (*S*)], 3.75 (m, 1 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.4 (d, $^1J_{C,C}$ = 33.8 Hz, C-8) ppm.

2-Methylpropionic Acid (21): Hydrogen peroxide solution (30 %, 2.7 mL, 27.9 mmol) and lithium hydroxide monohydrate (268 mg, 11.2 mmol), dissolved in water (5 mL), were added successively to a solution of **20** (1.38 g, 5.58 mmol) in THF (50 mL) and water (25 mL) at 0 °C. After 2 h, sodium sulfite (6.5 g) in water (25 mL) was added and the solution stirred at 0 °C for a further 15 min. The solution was adjusted to pH = 9−10 with satd. sodium hydrogencarbonate solution, the THF evaporated and the residual aqueous solution extracted with dichloromethane (2 × 50 mL). The organic extracts were dried with $MgSO_4$ and the solvents evaporated to yield (4*S*)-4-benzyloxazolidin-2-one (490 g, 97 %). The aqueous solution was acidified to pH = 1−2 with 1 M sulfuric acid and extracted with diethyl ether (3 × 125 mL). The combined extracts were dried with $MgSO_4$ and the solvents evaporated to yield 2-methylpropanoic acid (490 mg, quantitative) as a colourless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 (d, $^3J_{H,H}$ = 7.0 Hz, 6 H, 3-H), 2.58 (qq, $^3J_{H,H}$ = 7.0 Hz, 1 H, 2-H) ppm ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.6 (C-3), 33.7 (C-2), 183.1 (C-1) ppm.

[^{13}C]-(2*S*)-Methylpropionic Acid (21a): The procedure was successfully reproduced starting with (4*S*)-4-benzyl-3-[(2*S*)-2-methylpropionyl]oxazolidin-2-one (4.0 g, 16.1 mmol) giving [(2*S*)-^{13}C]methylpropionic acid as product (1.4 g, near quantitative). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 [dd, $^3J_{H,H}$ = 7.0, $^1J_{C,H}$ = 127.9 = Hz, 3 H, 3-H (^{13}C)], 1.19 (dd, $^3J_{H,H}$ = 7.0, $^3J_{C,H}$ = 5.2 Hz, 3 H, 3-H), 2.58 (dqq, $^3J_{H,H}$ = 7.0, $^2J_{C,H}$ = 2.4 Hz, 1 H, 2-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 33.7 (d, $^1J_{C,C}$ = 34.5 Hz, C-2) ppm.

2-Methyl-1-propanol: 2-Methylpropionic acid (1.27 g, 14.4 mmol), dissolved in dry ether (10 mL) was added to a slurry of $LiAlH_4$ (2.7 g, 72 mmol) in dry ether (25 mL) at 0 °C. After 6 h, the reaction was stopped with water, and the mixture was extracted with diethyl ether, washed with sodium hydrogencarbonate and brine, dried with $MgSO_4$ and the solvents were evaporated in vacuo to yield 2-methyl-1-propanol as a colourless liquid (853 mg, 80 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91 (d, $^3J_{H,H}$ = 6.7 Hz, 6 H, 3-H), 1.75 (tqq, $^3J_{H,H}$ = 6.7, $^3J_{H,H}$ = 6.5 Hz, 1 H, 2-H), 3.37 (d, $^3J_{H,H}$ = 6.5, 2 H, 1-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.6 (C-3), 30.1 (C-2), 68.7 (C-1) ppm. The procedure was successfully reproduced starting with [(2*S*)-^{13}C]methylpropionic acid (1.4 g, 16.1 mmol) giving [(2*S*)-^{13}C]methyl-1-propanol (1.1 g, 87 %) as product. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91 (dd, $^3J_{H,H}$ = 6.7, $^1J_{C,H}$ = 104.3 Hz, 3 H, 3-H (^{13}C)], 0.91 (dd, $^3J_{H,H}$ = 6.7, $^3J_{C,H}$ = 5.3 Hz, 3 H, 3-H), 1.75 (dtqq, $^3J_{H,H}$ = 6.7, $^3J_{H,H}$ = 6.5, $^2J_{C,H}$ = 3.6 Hz, 1 H, 2-H), 3.37 (dd, $^3J_{H,H}$ = 6.5, $^3J_{C,H}$ = 3.8 Hz, 2 H, 1-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 30.6 (d, $^1J_{C,C}$ = 34.5 Hz, C-2) ppm.

1-Iodo-2-methylpropane (22): Iodine (1.3 g, 5.0 mmol) was added to a solution of triphenylphosphane (1.31 g, 5.0 mmol) in nitrobenzene (25 mL). The brown colour persisted and an orange solid precipitated after ca. 10 min. A solution of 2-methyl-1-propanol (370 mg, 5.0 mol) in nitrobenzene (1 mL) was added followed by quinoline (1.2 mL, 10 mmol) and the reaction mixture was stirred overnight. Distillation of the reaction mixture occurred at 50 °C in 2 h to a cold trap (−196 °C) under reduced pressure (< 1.0 Torr). 1-Iodo-2-methylpropane was collected in 74 % yield (1.5 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.00 (d, $^3J_{H,H}$ = 6.6 Hz, 6 H, 3-H), 1.73 (m, 1 H, 2-H), 3.14 (d, $^3J_{H,H}$ = 5.9 Hz, 2 H, 1-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.4 (C-3), 22.6 (C-3), 30.4 (C-2) ppm.

[2-^{13}C]-1-Iodo-2-methylpropane (22a): The procedure was successfully reproduced starting with [(2*S*)-^{13}C]methylpropanol (1.1 g, 16 mmol) giving [(2*S*)-^{13}C]-1-iodo-2-methylpropane (2.1 g, 74 %) as product. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.00 (dd, $^3J_{H,H}$ = 6.6, $^1J_{C,H}$ = 125.8 Hz, 3 H, 3-H) 1.00 (dd, $^3J_{H,H}$ = 6.6, $^3J_{C,H}$ = 5.0 Hz, 3 H, 3-H), 1.73 (m, 1 H, 2-H), 3.14 (dd, $^3J_{H,H}$ = 5.9, $^3J_{C,H}$ = 4.3 Hz, 2 H, 1-H) ppm.

***tert*-Butyl *N*-Diphenylmethylidene-2-(2-methylpropyl)glycinate (23):** 1-Iodo-2-methylpropane (373 mg, 2.0 mmol) was added to a mixture of *tert*-butyl *N*-(diphenylmethylene)glycinate (500 mg, 1.69 mmol) and *O*-allyl-*N*-(9-anthracenylmethyl)cinchonidium bromide (200 mg, 0.34 mmol) in CH_2Cl_2 (8 mL). The reaction mixture was then cooled to −50 °C, and BTPP (2.5 mL, 8.5 mmol) was added dropwise. After 24 h, the solvent was evaporated in vacuo and the residue was purified by flash chromatography on silica gel (PE/EtOAc, 12:1) yielding the title compound as a yellowish oil (455 mg, 76 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.68 [d, $^3J_{H,H}$ = 6.5 Hz, 3 H, 5-H (*S*)], 0.85 [d, $^3J_{H,H}$ = 6.6 Hz, 3 H, 5-H (*R*)], 1.44 (s, 9 H, 8-H), 1.60 (m, 1 H, 4-H), 1.60 (m, 1 H, 3-H), 1.73 (ddd, 1 H, $^3J_{H,H}$ = 8.8, $^3J_{H,H}$ = 5.2, $^2J_{H,CH}$ 13.3 Hz, 3-H), 1.85 (ddd, 1 H, $^3J_{H,H}$ = 4.9, $^3J_{H,H}$ = 8.4, $^2J_{H,H}$ = 13.3 Hz, 3-H), 3.95 (dd, $^3J_{H,H}$ = 4.9, $^3J_{H,H}$ = 8.7 Hz, 1 H, 2-H), 7.4 (m, 10 H, 2 × Ph) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.7 [C-5 (*S*)], 23.2 [C-5 (*R*)], 24.7 (C-4), 28.0 (C-8), 42.7 (C-3), 64.6 (C-2), 80.7 (C-7), 127−139 (Ar), 169.7 (C-6), 172.0 (C-1) ppm.

***tert*-Butyl [^{13}C]-*N*-Diphenylmethylidene-2-(2-methylpropyl)glycinate (23a):** The procedure was successfully reproduced starting with [(2*S*)-^{13}C]-1-iodopropane (2.1 g, 11.4 mmol), *tert*-butyl *N*-(diphenylmethylene)glycinate (3.0 g, 10.4 mmol), catalyst (600 mg, 2.0 mmol) and BTPP (15.9 mL, 52 mmol) in CH_2Cl_2 (50 mL) giving **23a** (2.7 g, 75.6 %) as product. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.50−1.03 [2 dd, 6 H, 5-H (R), 5-H (*S*)], 1.60 (m, 1 H, 4-H), 1.73 (m, 1 H, 3-H), 1.85 (m, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.8 (d, $^1J_{C,C}$ = 35.0 Hz, C-4) ppm.

(2*S*)-Leucine (3): 6N HCl (10 mL) was added to **21** (1 g, 2.8 mmol) and the mixture was refluxed for 6 h. The mixture was then diluted with water and extracted with diethyl ether to remove the organic waste. Water was evaporated in vacuo and the residue lyophilized

three times. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.98 (d, $^3J_{H,H}$ = 3.8 Hz, 3 H, 6-H), 1.00 (d, $^3J_{H,H}$ = 3.6 Hz, 5-H), 1.73 (m, 1 H, 4-H), 1.83 (m, 2 H, 3-H), 3.94 (d, $^3J_{H,H}$ = 5.6, $^3J_{H,H}$ = 8.0 Hz, 1 H, 2-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.7 (C-5), 22.4 (C-6), 24.5 (C-4), 39.9 (C-3), 52.8 (C-2), 174.0 (C-1) ppm.

[5-^{13}C]-(2*S*,4*R*)-Leucine (3a): The procedure was successfully reproduced starting with **23a** (2.7 g, 7.6 mmol) giving [5-^{13}C]-(2*S*,4*R*)-leucine (1.0 g, quantitative) as product. The yield after two recrystallizations was 0.7 g (70 %), *ee* 99 %. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.98 (dd, 3 H, $^3J_{H,H}$ = 6.7, $^3J_{C,H}$ = 3.0 Hz, 6-H), 1.00 (dd, $^3J_{H,H}$ = 6.7, $^1J_{C,H}$ = 125.3 Hz, 3 H, 5-H), 1.75 (m, 1 H, 4-H), 1.83 (m, 2 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.5 (d, $^1J_{C,C}$ = 34.5 Hz, C-4) ppm.

Cbz-*tert*-Butyl Glycinate (28): Glycine (3 g, 40 mmol) was dissolved in an excess of 10 % Na_2CO_3 solution to which was added benzyl chloroformate (11.4 mL, 2 equiv.), dissolved in THF (25 mL). The mixture was stirred at room temp. for 4 h and subsequently extracted with ethyl acetate (3 × 50 mL) to remove excess benzyl chloroformate. The aqueous layers were concentrated in vacuo to give a white solid. The solid was redissolved in water (50 mL) and the pH was brought to 1 using 1 M HCl, after which the solution was extracted with ethyl acetate (3 × 50 mL) to give *N*-Cbz-glycine (5.81 g, 76 %) The protected glycine was dissolved in dimethylacetamide (75 mL) in the presence of benzyltriethylammonium chloride (1 equiv., 7.43 g). Dried K_2CO_3 (10 g) was added, followed by *tert*-butyl bromide (25 equiv., 90 g) and the mixture was stirred at 55 °C for 24 h. After cooling, 250 mL cold water was added and the resulting oil was extracted with ethyl acetate to give after concentration **28** (71 %, 5.37 g); **28** was dissolved in ethanol (50 mL) and the solution was purged with argon. Subsequently, 10 % Pd/C was added to the mixture and hydrogen gas was bubbled through until TLC indicated complete removal of the Z-protecting group. The solution was purged with argon and filtered through Celite under argon. The Celite was rinsed with ethanol (50 mL) and the solvent was evaporated. The raw product was redissolved in ethyl acetate (50 mL) and extracted with 1 M HCl (2 × 50 mL). The water layers were collected and brought to pH = 12 with 50 % KOH solution. After extraction with ethyl acetate (2 × 50 mL), the organic layers were combined, dried with $MgSO_4$ and the solvents evaporated to give **25** in 92 % yield (2.6 g). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.47 (s, 9 H), 3.31 (s, 2 H, 2-H) ppm.

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