Synthesis of (ε-^{13}C-,ε-^{15}N)-Enriched L-Lysine − Establishing Schemes for the Preparation of All Possible ^{13}C and ^{15}N Isotopomers of L-Lysine, L-Ornithine, and L-Proline

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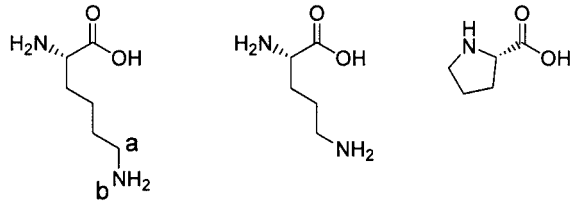
In this paper we describe a simple synthetic strategy that, with the right rational adaptation, gives direct access to any ^{13}C/^{15}N isotopomer of L-glutamate, L-ornithine, L-proline, L-lysine, and L-α amino adipic acid. This strategy also allows access to nonproteinogenic amino acids like L-citrulline in high yields and optical purity.

Introduction

Rhodopsin is the photosensitive protein of the rod photoreceptor in the vertebrate retina that mediates dim light vision. Rhodopsin represents a paradigm for the large and diverse family of G-protein coupled membrane receptors (GPCR's).[1] Lysine-296 in the active site of rhodopsin connects the 11-Z protonated Schiff base of retinal to the protein.[2] In order to study the role of this residue in the rhodopsin photoreceptor process with ^{1}H and ^{13}C solid-state NMR techniques we needed access to (ε-^{13}C,ε-^{15}N)lysine (Figure 1, compound **1a**). Lysine is also an essential amino acid in human (and animal) nutrition, and access to the set of site-directed isotopomers of lysine is essential to elucidate the metabolic role of this amino acid by mass-spectral techniques.



1 **2** **3**

1a: a = ^{13}C, b = ^{15}N

Figure 1. L-lysine (**1**), L-ornithine **2**) and L-proline (**3**)

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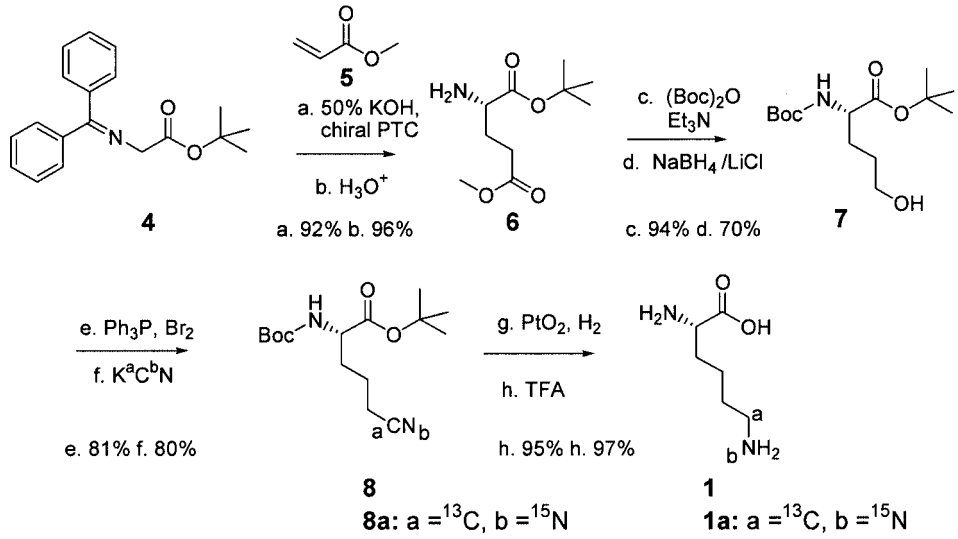
In this paper we describe a modular strategy that is able to give access to any site-directed isotopically labeled L-lysine, L-ornithine and L-proline, with the use of commercially available stable isotopically enriched synthons or synthons that are available in any site-directed enriched form by strategies described before by us.[3,4] For the ^{1}H, ^{13}C, and ^{15}N MAS NMR study we need, next to ($^{13}C_{20}$)-11-(*Z*)-retinal, also (ε-^{15}N,ε-^{13}C)lysine.[5] The one-step synthesis of the protected L-glutamate ester by the O′ Donnell method[6] has been published for the natural abundance materials.[7] In our group, we have worked out schemes to prepare the required synthons in any site-directed isotopomeric form.[3,4] Glutamic acid has the complete carbon skeleton of other proteinogenic amino acids such as proline and glutamine. For the preparation of lysine, only one additional carbon and nitrogen atoms are needed. The basis of using glutamic acid as a building block for the preparation of the aforementioned amino acids in each site-directed isotopically enriched form is the possibility of the selective conversion of the 5-carboxylic acid ester group into other functional groups. The protected glutamate obtained by the O′Donnell method has proved to be the perfect intermediate in this synthesis.

Results and Discussion

Synthetic Strategy

In Scheme 1, the strategy to prepare any site-directed enriched isotopomer of L-lysine **1** is indicated. The first step is the O′ Donnell coupling of the protected glycine derivative[3] **4** with methyl acrylate **5**, which can be prepared in any isotopomeric form, as has been previously published by our group.[4] The product of the coupling after treatment



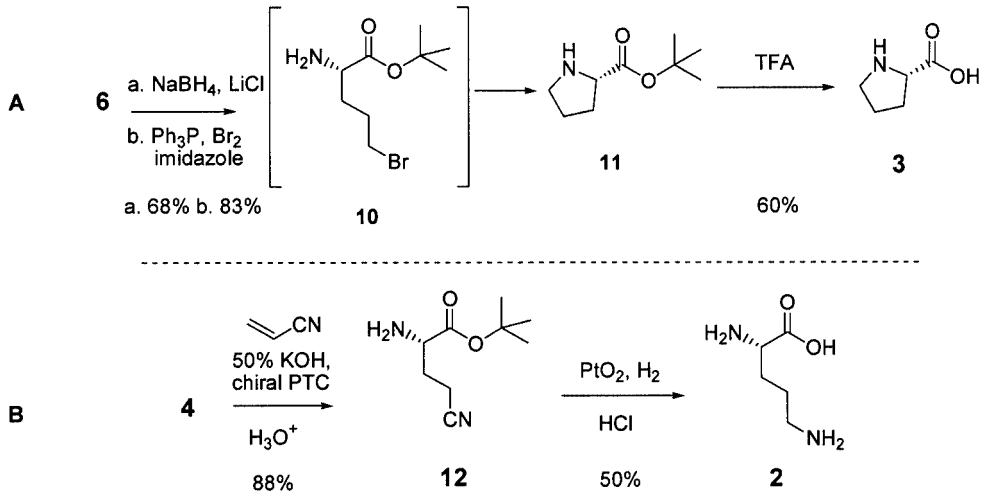
Scheme 1. The synthesis of L-lysine (**1**) starting from the achiral scaffold *N*-diphenylmethylene glycine *tert*-butyl ester (**4**)

with aqueous citric acid gives the *tert*-butyl, methyl ester of glutamic acid **6**. The free amino group in **6** is subsequently converted into the Boc derivative in 85% yield. This fully protected glutamate is then treated with $NaBH_4$/LiCl in ethanol at 0 °C.[8] This reagent is a mild and selective reducing agent, capable of selectively reducing the methyl ester to the alcohol **7**, without concomitant reduction of the *tert*-butyl ester. Conversion of the alcohol function in **7** with triphenylphosphane and bromine in dichloromethane (DCM) gives the corresponding bromide in 81% yield. Subsequent treatment of the bromide with KCN in refluxing acetone gives the protected adiponitrile **8** in 80% yield. Catalytic reduction with Adam's catalyst (PtO_2) in acidified ethanol and subsequent deprotection with 10% trifluoroacetic acid leads to L-lysine **1**, with all the characteristics of the authentic material. With the use of $K^{13}C^{15}N$, **8a** can be prepared in 76% yield from the bromide. Reduction of the labeled nitrile and subsequent deprotection gives (ε-^{13}C,ε-^{15}N)L-lysine in 91% yield. Introducing $K^{13}C^{15}N$ in the final steps gives (ε-^{13}C,ε-^{15}N)lysine in 69% yield based on $K^{13}C^{15}N$. It is clear from Scheme 1 that L-lysine can be prepared in any isotopomeric form.

Before we developed the present synthetic scheme we have prepared **1a** by the O′ Donnell method using (^{13}C,^{15}N)4-iodobutyronitrile. Labeled iodobutyronitrile was prepared by S_N2-substitution of the iodo-substituent of 3-chloro-1-iodopropane with $K^{13}C^{15}N$, followed by treatment of the resulting chlorobutyronitrile with KI in refluxing acetone. Based on this method, the overall yield of **1a** from $K^{13}C^{15}N$ is 37%. This method does allow access to the full set of isotopomers,[9] but gives a lower yield based on the incorporated isotopes, hence the development of the synthesis presented in Scheme 1. In Scheme 1, the α-aminoadiponitrile derivative **8** is the intermediate that can be easily hydrolyzed to α-aminoadipic acid.[10] α-Aminoadipic acid is an essential intermediate in the biosynthesis of L-lysine in microorganisms,[11] and access to the full set of isotopomers is beneficial to metabolic research.

For the synthesis of proline **3**, the internal alkylation reaction of the α-amino group with $C^{\delta}-Br$ leads directly to L-proline (**3**) (Scheme 2). The reduction of **6** with $NaBH_4$/LiCl to the amino alcohol is directly followed by treatment with the adduct of triphenylphosphane and bromine in dichloromethane in the presence of imidazole as proton scavenger. The resulting bromide **10** reacts intramolecularly with the amino group to form the proline *tert*-butyl ester. Removal of the ester group with 10% TFA in DCM gives L-proline (**3**). This scheme thus gives access to all isotopomers of L-proline (**3**) in a simpler and more convergent fashion than existing methods.[12]

The simple catalytic reduction of adiponitrile **8** to form the protected lysine motivated us to prepare L-ornithine in a similar way through reduction of glutaronitrile **12**. The protected glycinate **4** was treated under O′ Donnell conditions with acrylonitrile, which can be prepared in all isotopomeric forms,[13] to form the protected glutaronitrile derivative, which upon treatment with aqueous citric acid gives **12**. Catalytic reduction and acid-mediated deprotection leads to L-ornithine **2** with the analytical properties of the authentic material. Access to all stable ^{13}C and ^{15}N isotopomers of ornithine allows preparation of the isotopomers of other amino acids that share the same carbon skeleton. In this way, starting from ornithine, arginine and citrulline can easily be prepared in a few simple steps. Treatment of L-ornithine with the cyanate anion gives citrulline.[14] Earlier, our group prepared all isotopomers of cyanate and prepared site-directed ^{15}N- and ^{13}C-enriched urea derivatives from primary amines.[15] The conversion of L-ornithine into L-arginine with ^{13}C and ^{15}N isotopes has also been published.[16,17] The ability to prepare all ^{15}N and ^{13}C isotopomers of the three amino acids ornithine, arginine, and citrulline will be a boon to the metabonomics



Scheme 2. **A** − The synthesis of L-proline (**3**) starting from chiral *tert*-butyl methyl glutamate (**6**); **B** − the synthesis of L-ornithine (**2**) starting from achiral glycinate **4**

studies of the vertebrate urea-cycle since all intermediates of this cycle can now be prepared easily in any desired isotopomeric form.

Conclusion

In this paper we describe the modular and convergent strategy to prepare L-lysine (**1**), L-ornithine (**2**), and L-proline (**3**) in an optically pure form such that all site-directed ^{13}C and ^{15}N isotopomers can easily be prepared by the alkylation of a protected glycine derivative using Michael-type reactions with methyl acrylate and acrylonitrile in the presence of a chiral phase-transfer catalyst. After the reaction, the optically active catalyst can easily be isolated, ready to be reused. With this method, no difficult separation of the product from an optically active scaffold is needed.[18] We feel that this scheme can easily be adapted to the synthesis of a whole class of new nonproteinogenic α-amino acids in high optical purity.

Experimental Section

General Remarks: ^{1}H NMR spectra were recorded with a Jeol FX-200, a Bruker DPX-300 or a Bruker DPX 400 spectrometer using tetramethylsilane (TMS: δ = 0 ppm) or water (H_2O: δ = 4.8 ppm) as an internal standard. ^{13}C noise-decoupled NMR spectra were recorded with a Jeol FX-200 at 50.1 MHz, a Bruker DPX-300 spectrometer at 75.5 MHz, and a Bruker DPX-400 at 100.7 MHz, using $CDCl_3$ (δ = 77 ppm), $(CD_3)_2CO$ (δ = 206 ppm), or TSP [3-(trimethylsilyl)tetradeuteriopropionic acid sodium salt, δ = 0 ppm] as internal standard. A saturated solution of ammonium nitrate was used as an external standard ($^{15}NH_4NO_3$ δ = 22.3 ppm relative to NH_3 (l) δ = 0.0 ppm) for the ^{15}N NMR spectra. All spectra were recorded in $CDCl_3$, except where noted otherwise. Column chromatography was performed on Merck silica gel 60 (0.040−0.063 mm 230−400 mesh), and spots on the thin-layer chromatograph were detected with UV light, $KMnO_4$ solution spraying, ninhydrin staining (0.2% in ethanol) or staining with a mixture of 4,4′-methylenebis(*N*,*N*-dimethylaniline) and ninhydrin (TDM staining). Chiral HPLC was performed with a Chiralcel ODH column (25 cm) using hexane and 2-propanol (*i*PrOH) as solvent system. Dry diethyl ether (ether, Et_2O) was obtained by distilling from P_2O_5. Dry petroleum ether (PE, boiling range 40−60 °C) and dry dichloromethane (DCM) were obtained by distilling from CaH_2. All commercially available chemicals were purchased from Sigma−Aldrich, Across or Fluka. All chemicals were used without further purification, unless stated otherwise. (^{13}C,^{15}N)potassium cyanide (> 99% isotope-enriched) was purchased from Cambridge Isotope Laboratories.

***N*-(Benzophenoneimine) of 1-*tert*-*Buty*l 5-Methyl Glutamate:** Methyl acrylate (**5**) (1.98 mL, 22 mmol) dissolved in toluene (3 mL) was added dropwise to a solution of **4** (5 g, 17 mmol) in toluene (15 mL), and a 50% KOH solution (1/3 volume of toluene, 6 mL) with *O*-allyl-*N*-(9-anthracenyl)methylcinchonidium bromide (0.05 equiv.) was added as chiral phase-transfer catalyst. The solution was stirred very vigorously for 14 h. The mixture was then diluted with 10 mL water and extracted with dichloromethane (3 × 20 mL). The collected organic layers were dried with $MgSO_4$, and the solvents evaporated. The product was further purified over a silica column (PE/Et_2O, 90:10) to yield the product in 92% (5.97 g, 15.6 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.48 (s, 9 H, *t*Bu), 2.23 (m, 2 H, 4-H), 2.41 (m, 2 H, 3-H), 3.56 (s, 3 H, OMe), 4.01 (dd, $^3J_{H,H}$ = 7.28, $^3J_{H,H}$ = 5.50 Hz, 1 H, 2-H), 7.14−7.66 (m, 10 H, arom.) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 27.77 (*t*Bu), 28.4 (C-4), 30.21 (C-3), 51.18 (OMe), 64.54 (C-2), 80.84 (*t*Bu), 127.52−139.19 (aromatic), 170.44 (C-1 + CN), 173.20 (C-5) ppm.

1-*tert*-Butyl 5-Methyl Glutamate (6): The alkylated protected glycinate (5.97 g, 15.6 mmol) was dissolved in THF (10 mL) and stirred with a 10% citric acid solution (20 mL) for one night. TLC showed the complete disappearance of the starting material, and the mixture was extracted twice with ether (25 mL). The water layer was then brought to pH 12 and extracted with ethyl acetate (2 × 30 mL) to give, after evaporation, the product **6** in 96% yield (3.26 g). ^{1}H NMR (300 MHz): δ = 1.47 (s, 9 H, *t*Bu), 1.81 (m, 1 H, 3-H), 2.03 (m, 1 H, 3-H), 2.36 (dd, 2 H, 4-H), 3.34 (dd $^3J_{H,H}$ = 5.3, $^3J_{H,H}$ = 8.2 Hz, 1 H, 2-H), 3.68 (s, 3 H, OMe) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 27.35 (*t*Bu), 29.60 (C-4) 29.80 (C-3), 50.90 (C-2), 53.65 (OMe), 80.44 (*t*Bu), 172.95 (C-1), 174.11 (C-5) ppm.

1-*tert*-Butyl 5-Methyl *N*-(Boc)glutamate: The amino ester was subsequently dissolved in DMF (10 mL) and triethylamine (1 equiv., 2.1 mL) was added, followed by di-*tert*-butyl dicarbonate (1.1 equiv., 3.53 mL), and the mixture was stirred until TLC showed the completion of the reaction. The DMF was evaporated, and ethyl acetate (25 mL) was added. The organic layer was washed with $KHSO_4$ solution (pH 2, 3 × 15 mL), water, brine, and dried with $MgSO_4$. The raw product was purified over a short silica column (PE/Et_2O, 80:20). The yield was 94% (4.48 g, 14.1 mmol). ^1H NMR (300 MHz): δ = 1.44 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 1.91 (m, 1 H, 3-H), 2.16 (m, 1 H, 3-H), 2.34 (m, 2 H, 4-H), 3.68 (s, 3 H, OMe), 4.19 (m, 1 H, 2-H), 5.10 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, N-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 27.87 (*t*Bu), 27.96 (C-4) 28.20 (*t*Bu), 30.00 (C-3), 51.61 (OMe), 53.31 (C-2), 79.63 (*t*Bu), 82.04 (*t*Bu), 155.27 (NCO), 171.20 (C-1), 173.20 (C-5) ppm.

***tert*-Butyl *N*-Boc-2-amino-5-hydroxypentanoate (7):** $NaBH_4$ (4 equiv., 278 mg) was added to a suspension of LiCl (4 equiv., 311 mg) in THF/EtOH (10 mL, 1:1), and the mixture was stirred for 10 min. After cooling to 0 °C, a solution of the glutamate (1.8 mmol, 580 mg) dissolved in THF/EtOH (5 mL) was added dropwise. The reaction mixture was stirred overnight, and the temperature was allowed to rise to room temperature. Water was added (30 mL), and the reaction mixture was extracted with EtOAc (3 × 30 mL). The collected organic layers were extracted with brine and dried with $MgSO_4$. Subsequently, the solvents were evaporated, and the raw product was purified using column chromatography (15% to 25% EtOAc in PE) to give **7** in 70% yield (361 mg, 1.26 mmol). ^1H NMR (300 MHz): δ = 1.47 (2*s, 18 H, 2**t*Bu), 1.54−1.95 (m, 4 H, 3-H, 4-H), 3.66 (m, 2 H, 5-H), 4.20 (m, 1 H, 2-H), 5.15 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 27.88 (*t*Bu), 27.90 (C-4) 28.23 (*t*Bu), 29.53 (C-3), 53.55 (C-2), 61.95 (C-5), 79.63 (*t*Bu), 81.80 (*t*Bu), 155.88 (NCO), 171.88 (C-1) ppm.

***tert*-Butyl *N*-Boc-2-amino-5-cyanopentanoate (8):** Triphenylphosphane (0.66 g, 2 equiv.) was dissolved in dry dichloromethane (5 mL), the solution was cooled to 0 °C and stirred under a dry nitrogen while bromine (128 μL, 1.99 equiv.) dissolved in DCM (2 mL) was added dropwise. After 20 minutes, a mixture of **7** (361 mg, 1.26 mmol) and imidazole (0.18 g, 2 equiv.) dissolved in DCM (5 mL) was slowly added to the pale yellow solution. After 1 min, a white solid became visible. The reaction mixture was stirred for another 2 h while the temperature was maintained at 0 °C. Subsequently, the solids were filtered off, and the solvent was evaporated. The resulting product was redissolved in DCM (10 mL) and the solution filtered quickly over a glass-filter filled with some silica. The silica was rinsed with EtOAc (50 mL), all solutions were combined. After evaporation of the solvents the bromide was obtained in 81% yield (0.35 g, 1.0 mmol). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 1.78−199 (m, 4 H, 3-H,4-H), 3.41 (m, 2 H, 5-H), 4.18 (m, 1 H, 2-H), 5.31 (d, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 28.0 (*t*Bu), 28.3 (*t*Bu), 30.3 (C-3), 31.6 (C-4), 33.0 (C-5), 53.2 (C-2), 79.8 (*t*Bu), 82.14 (*t*Bu), 155.3 (CO), 171.5 (C-1) ppm. The bromide (0.35 g, 1.0 mmol) was dissolved in ethanol (30 mL), and after adding potassium cyanide (1.1 equiv., 74 mg) dissolved in ethanol/water (5 mL, 90:10 v/v), the mixture was refluxed for 6 h. The solution was cooled to room temperature and concentrated to a quarter of its volume, after which the precipitate was filtered off, and the solution was extracted with dichloromethane (2 × 40 mL). Subsequently, the collected organic layers were washed with water (20 mL). The solution was dried with $MgSO_4$, and after removing the solvent in vacuo, the raw product was purified by column chromatography (PE/Et_2O, 85:15) to yield **8** in 80% yield (237 mg, 0.8 mmol). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 1.63 (m, 2 H, 4-H), 1.72 (m, 2 H, 3-H), 2.40 (m, 2 H, 5-H), 4.20 (m, 1 H, 2-H), 5.05 (m, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 16.44 (C-5), 21.26 (C-4), 28.0 (*t*Bu), 28.3 (*t*Bu), 31.12 (C-3), 52.8 (C-2), 79.5 (*t*Bu), 82.0 (*t*Bu), 118.70 (C-6), 155.2 (CO), 171.5 (C-1) ppm.

***tert*-Butyl (5-^{13}C,^{15}N)-*N*-Boc-2-amino-5-cyanopentanoate (8a):** Prepared as described for **8**. Yield: 260 mg, 0.87 mmol **8a**. Starting from bromide (0.36 g, 1.04 mmol) and $K^{13}C^{15}N$ (77 mg, 1.1 equiv.). The yield was 76% based on the labeled KCN, and 84% based on the bromide. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 1.63 (m, 2 H, 4-H), 1.72 (m, 2 H, 3-H), 2.40 (m, 2 H, 5-H), 4.20 (m, 1 H, 2-H), 5.05 (m, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 16.44 (d, $^1J_{C,C}$ = 56.6 Hz, C-5), 21.26 (d, $^2J_{C,C}$ = 2.6 Hz, C-4), 28.0 (*t*Bu), 28.3 (*t*Bu), 31.12 (C-3), 52.8 (C-2), 79.5 (*t*Bu), 82.0 (*t*Bu), 118.70 (d, 1.6, $^1J_{C,N}$ = 16.8 Hz, C-6), 155.2 (CO), 171.5 (C-1) ppm.

L-Lysine·TFA (1): The purified protected nitrile (237 mg, 0.8 mmol) was dissolved in 2-propanol (30 mL), together with concentrated HCl (2 mL) and a catalytic amount of platinum oxide. Using a Parr-apparatus, the mixture was then shaken vigorously under 50 psi H_2 for 16 h. Thereafter, the mixture was filtered through celite to remove the platinum catalyst and concentrated in vacuo to give the free amine in 95% yield (226 mg, 0.76 mmol). The raw product was redissolved in a mixture of 10% trifluoroacetic acid in dry DCM (20 mL) and stirred overnight. The mixture was subsequently extracted with water (3 × 15 mL), and the combined water layers were evaporated, and the sample was lyophilized to give the lysine·TFA salt **1** in 97% yield (190 mg, 0.73 mmol). ^1H NMR (400 MHz, D_2O): δ = 1.55 (m, 2 H, 4-H), 1.75 (m, 2 H, 3-H), 1.99 (m, 2 H, 5-H), 3.2 (dt, $^3J_{H,H}$ = 7.6 Hz, 2 H, 6-H), 3.7 (dd, $^3J_{H,H}$ = 6.4 Hz, 1 H, 2-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 22.0 (C-4), 26.83 (C-5), 29.8 (C-3), 39.7 (C-6), 53.7 (C-2), 117.1 (q, CF_3), 163.6 (q, CO), 175.3 (C-1) ppm.

(ε-^{13}C,^{15}N)-L-Lysine (1a): Prepared as described above. The yield was 91% (220 mg, 0.84 mmol) starting from **8a**. ^1H NMR (400 MHz, D_2O): δ = 1.55 (m, 2 H, 4-H), 1.75 (m, 2 H, 3-H), 1.99 (m, 2 H, 5-H), 3.2 (dt, $^3J_{H,H}$ = 7.6, $^1J_{C,H}$ = 143 Hz, 2 H, 6-H), 3.7 (dd, $^3J_{H,H}$ = 6.4 Hz, 1 H, 2-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 22.0 (s, C-4), 26.83 (d, $^1J_{C,C}$ = 35.2 Hz, C-5), 29.8 (d, $^3J_{C,C}$ = 5.3 Hz, C-3), 39.7 (d, $^1J_{C,N}$ = 4.9 Hz, C-6), 53.7 (C-2), 175.3 (C-1) ppm.

(4-^{13}C,^{15}N)-1-Chlorobutyronitrile: A mixture of 1-bromo-3-chloropropane (22.0 g, 14.0 mL, 140 mmol) and (^{13}C,^{15}N)potassium cyanide (2.39 g, 35.6 mmol) dissolved in ethanol/water (100 mL, 90:10 v/v) was refluxed for 3 h. The solution was cooled to room temperature, the precipitate was filtered off, and the solution was extracted with dichloromethane and washed with water (3 ×). After removing the solvent in vacuo, the solution was further purified by vacuum distillation to remove the excess 1-bromo-3-chloropropane. The product (a yellow oil) was obtained in 76% yield (2.92 g, 27 mmol). ^1H NMR (400 MHz, $CDCl_3$): δ = 2.11 (ddt, $^3J_{H,H}$ = 6.0, $^3J_{H,H}$ = 7.0, $^3J_{C,H}$ = 6.6 Hz, 2 H, 2-H), 2.57 (ddt, $^3J_{H,H}$ = 7.0, $^2J_{C,H}$ = 10, $^3J_{N,H}$ = 1.6 Hz, 2 H, 3-H), 3.67 (t, $^3J_{H,H}$ = 6.0 Hz, 2 H, 1-H), ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.59 (dd, $^1J_{C,C}$ = 56.7, $^2J_{C,N}$ = 3.0 Hz, C-3), 28.9 (d, $^2J_{C,C}$ = 2.5 Hz, C-2), 42.5 (d, $^3J_{C,C}$ = 3.8 Hz, C-1), 118.4 (d, $^1J_{C,N}$ = 16.9 Hz, C-1) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ = 248.6 (dt, $^3J_{N,H}$ = 1.6, $^1J_{C,N}$ 16.8 Hz, ^{15}N) ppm.

(4-^{13}C,^{15}N)-1-Iodobutyronitrile: 1-Chlorobutyronitrile (2.92 g, 27 mmol) and NaI (3 equiv.) were dissolved in warm acetone

(150 mL) and refluxed for 24 h, during which the mixture gradually became yellow. The mixture was cooled to 20 °C. After distilling off the solvent, the residue was diluted with water and extracted with dichloromethane. The organic layer was successively washed with 1 M Na_2SO_4 and dried with $MgSO_4$. The product was concentrated in vacuo and purified over a silica column (PE/Et_2O, 90:10) to give a colorless liquid (5.05 g, 19.4 mmol) in 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.09 (dt, $^3J_{H,H}$ = 7.0, $^3J_{H,H}$ = 6.0, $^3J_{C,H}$ = 6.6 Hz, 2 H, 2-H), 2.50 (ddt, $^2J_{C,H}$ = 9.7, $^3J_{H,H}$ = 6.8, $^3J_{N,H}$ = 1.8 Hz, 2 H, 3-H), 3.22 (t, $^3J_{H,H}$ = 6.4 Hz, 2 H, 1-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.3 (d, $^3J_{C,C}$ = 3.7 Hz, C-1), 17.8 (dd, $^1J_{C,C}$ = 55.7, $^2J_{C,N}$ 3.0 Hz, C-3), 28.1 (d, $^2J_{C,C}$ = 2.9 Hz, C-2) 117.8 (d, $^1J_{C,N}$ = 16.9 Hz, C-4) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ = 248.7 (dt, $^3J_{N,H}$ = 1.6, $^1J_{C,N}$ = 16.8 Hz, ^{15}N) ppm.

***tert*-Butyl (ε-^{13}C,ε-^{15}N)-2-(Benzhydrylideneamino)-5-cyanopentanoate:** 1-Iodobutyronitrile (5.05 g, 25.6 mmol) was added dropwise to a solution of *tert*-butyl *N*-(diphenylmethylene)glycinate (1.1 equiv., 8.2 g, 28.1 mmol) in toluene (40 mL) and 50% KOH (1/3 volume of toluene) in the presence of *O*-allyl-*N*-(9-anthracenyl)methylcinchonidium bromide (0.1 equiv.) as a phase-transfer catalyst. The solution was stirred very vigorously for 12 h. The mixture was then diluted with water, extracted with dichloromethane (3 × 20 mL), and dried with $MgSO_4$. The product was further purified over a silica column (PE/Et_2O, 90:10) to yield a mixture of unchanged 4-iodonitrile (which could be recovered and reused) and product in 50% yield (4.6 g, 12.8 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 (s, 9 H, *t*Bu), 1.65 (m, 2 H, 4-H), 1.98 (m, 2 H, 3-H), 2.31 (m, 2 H, 5-H), 3.94 (dd, $^3J_{H,H}$ = 5.0, $^3J_{H,H}$ = 7.1 Hz, 1 H, 2-H), 7.15−7.68 (m, 10 H, arom.) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 16.8 (dd, $^1J_{C,C}$ 55.98, $^2J_{C,N}$ 2.8 Hz, C-5), 21.9 (d, $^2J_{C,C}$ 2.46 Hz, C-4), 27.81 (*t*Bu), 32.2 (d, $^3J_{C,C}$ 3.68 Hz, C-3), 64.7 (C-2), 81.09 (*t*Bu), 117−139 (arom.) 119.4 (d, $^1J_{C,N}$ = 16.9 Hz, C-6), 170.44 (CN), 178.10 (C-1) ppm.

***tert*-Butyl (ε-^{13}C,ε-^{15}N)-2-Amino-5-cyanopentanoate:** The protected amino acid (4.6 g, 12.8 mmol) and unchanged 4-iodobutyronitrile were dissolved in THF (50 mL) and diluted with a 15% citric acid solution (20 equiv.). After stirring for 3 h, the mixture was diluted with water and extracted with diethyl ether to remove the benzophenone and the unchanged iodonitrile. The water phase was then neutralized with K_2CO_3 (pH 11−12) and extracted with ethyl acetate (3 × 20 mL). The combined organic phases were dried with $MgSO_4$ and concentrated in vacuo. The mixture was further purified over a silica column (PE/EtOAc, 85:15) to obtain the product in 96% yield (2.8 g, 12.2 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.47 (s, 9 H, *t*Bu), 1.64 (m, 1 H, 3-H), 1.8 (m, 3 H, 3-H + 4-H), 2.4 (m, 2 H, 5-H), 3.32 (m, 1 H, H-2) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 16.8 (dd, $^1J_{C,C}$ = 56.02, $^2J_{C,N}$ = 3.0 Hz, C-5), 21.76 (d, $^2J_{C,C}$ = 2.7 Hz, C-4), 27.75 (*t*Bu), 33.4 (d, $^3J_{C,C}$ = 1.6 Hz, C-3), 81.14 (*t*Bu), 119.27 (d, $^1J_{C,N}$ = 16.9 Hz, C-6), 174.5 (s, CO) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ = 246 (dt, $^1J_{C,N}$ = 16.93, $^3J_{H,N}$ = 1.61 Hz, ^{15}N) ppm.

(ε-^{13}C, ε-^{15}N)-L-Lysine *tert*-Butyl Ester: The purified nitrile (2.80 g, 12.3 mmol) was dissolved in 2-propanol (50 mL), together with concentrated HCl (3 mL) and a catalytic amount of platinum oxide. The mixture was then stirred vigorously under 50 psi H_2 for 16 h using a Parr-apparatus. Thereafter, the mixture was filtered through celite to remove the platinum catalyst, which was rinsed with 2-propanol (25 mL), and concentrated in vacuo to give the amine in 98% yield (2.4 g, 12 mmol). ^{1}H NMR (400 MHz, D_2O): δ = 1.40 (s, 9 H, *t*Bu), 1.45 (m, 2 H, 4-H), 1.65 (m, 2 H, 5-H), 1.85 (m, 2 H, 5-H), 2.9 (dt, $^3J_{H,H}$ = 7.5, $^1J_{C,H}$ = 143 Hz, 2 H, 6-H), 4.0 (m, 1 H, 2-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 22.16 (s, C-4), 27.04 (d, $^1J_{C,C}$ = 35.9 Hz, C-5), 27.80 (*t*Bu), 30.5 (d, $^3J_{C,C}$ = 5.3 Hz, C-3), 39.7 (d, $^1J_{C,N}$ = 4.9 Hz, C-6), 53.6 (s, C-2), 82.52 (*t*Bu), 173.0 (C-1) ppm.

(ε-^{13}C, ε-^{15}N)-L-Lysine·HCl (1a): L-lysine *tert*-butyl ester was dissolved in 6 M HCl (100 mL). The mixture was then refluxed for 24 h. After removing the solvent in vacuo, the yellow residue was taken up in water (100 mL), treated with a small amount of charcoal (Norit), and refluxed for 2 h to decolorize the solution. After filtering off the charcoal, the solution was concentrated in vacuo. Recrystallization from water/ethanol yielded L-lysine monohydrochloride as a white hygroscopic powder in 90% yield [1.95 g, 10.8 mmol, (37% yield based on $K^{13}C^{15}N$)]. $[\alpha]_D^{23}$ = +24.8 (*c* = 2, 6 N HCl), ref. $[\alpha]_D^{20}$ = +25.2 (*c* = 2, 6 N HCl). ^{1}H NMR (400 MHz, D_2O): δ = 1.55 (m, 2 H, 4-H), 1.75 (m, 2 H, 3-H), 1.99 (m, 2 H, 5-H), 3.2 (dt, $^3J_{H,H}$ = 7.6, $^2J_{C,H}$ = 143 Hz, 2 H, 6-H), 3.7 (dd, $^3J_{H,H}$ = 6.4 Hz, 1 H, 2-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 22.0 (s, C-4), 26.83 (d, $^1J_{C,C}$ = 35.2 Hz, C-5), 29.8 (d, $^3J_{C,C}$ = 5.3 Hz, C-3), 39.7 (d, $^1J_{C,N}$ = 4.9 Hz, C-6), 53.7 (C-2), 175.3 (C-1) ppm.

***tert*-Butyl 2-[(Benzhydrylidene)amino]-4-cyanobutanoate:** Acrylonitrile **5** (116 μL, 1.77 mmol) dissolved in toluene (2 mL) was added dropwise to a mixture of *N*-(diphenylmethylene)-*tert*-butyl glycinate (400 mg, 1.36 mmol) in toluene (4 mL) and 50% KOH (2 mL) with O-allyl *N*-9-anthracenyl methylcinchonidium bromide (41 mg, 0.05 equiv.) as a chiral phase-transfer catalyst. The solution was stirred very vigorously for 14 h. The mixture was then diluted with water (10 mL) and extracted with dichloromethane (3 × 10 mL). The collected organic layers were dried with $MgSO_4$, and the solvents evaporated. The product was further purified over a silica column (90:10, PE/ether) to give the product in 92% yield (434 mg, 1.25 mmol). *ee* = 91% (chiralcel ODH, 25 cm, hexane/*i*PrOH, 99.9:0.1 v/v, flow 0.8 mL/min, retention time: 19.05 min (*R*); 27.35 min (*S*). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, *t*Bu), 2.13−2.37 (m, 2 H, 3-H), 2.44−2.54 (m, 2 H, 4-H), 4.05 (dd, $^3J_{H,H}$ = 7.55, $^3J_{H,H}$ = 4.80 Hz, 1 H, 2-H), 7.17−7.68 (m, 10 H, aromatic) ppm. ^{13}C NMR (50.1 MHz, $CDCl_3$): δ 13.57 (C-3), 27.96 (*t*Bu), 29.39 (C-4), 63.69 (C-2) 81.76 (*t*Bu), 119.32 (C-5), 127.59−135.99 (arom.), 158.10 (CN), 169.76 (C-1) ppm.

Proline *tert*-Butyl Ester (11): $NaBH_4$ (4 equiv., 430 mg) was added to a suspension of LiCl (4 equiv., 483 mg) in THF/EtOH (10 mL, 1:1), and the mixture was stirred for 10 min. After cooling to 0 °C, a solution of **6** (2.7 mmol, 600 mg) dissolved in THF/EtOH (5 mL) was added dropwise. The reaction mixture was stirred overnight, and the temperature was allowed to rise to room temperature. A KOH solution (pH 13, 30 mL) was added, and the reaction mixture was extracted with EtOAc (3 × 30 mL). The collected organic layers were dried with $MgSO_4$. Subsequently, the solvents were evaporated, and the raw product was purified using column chromatography (15% to 35% EtOAc in PE) to give the product in 68% yield (340 mg, 1.9 mmol).

Triphenylphosphane (1.0 g, 2 equiv.) was dissolved in dry dichloromethane (5 mL), and the solution was cooled to 0 °C and stirred under dry nitrogen while bromine (193 μL, 1.99 equiv.) dissolved in DCM (2 mL) was added dropwise. After 20 minutes, a mixture of the alcohol (340 mg, 1.9 mmol) and imidazole (0.27 g, 2 equiv.) dissolved in DCM (5 mL) was slowly added to the pale yellow solution. After 1 min, a white solid became visible. The reaction mixture was stirred for another 3 h, while the temperature was maintained at 0 °C. Subsequently, a 0.1 M KOH solution (10 mL) and DCM (10 mL) were added, the organic layer was separated, and the solvent was evaporated. The resulting product was redissolved

in DCM (10 mL) and the solution filtered quickly over a glass-filter with some silica. The silica was rinsed with EtOAc (50 mL), all filtrates were combined and the solvents evaporated to give the proline *tert*-butyl ester in 83% yield (0.52 g, 1.57 mmol). ^{1}H NMR (200 MHz, D_2O/DCl): δ = 1.50 (s, 9 H, *t*Bu), 2.12 (m, 3 H, 3-H, 4-H), 2.43 (m, 1 H, 4-H), 3.42 (m, 2 H, 5-H), 4.35 (m, 1 H, H-2) ppm.

L-Proline·HCl (3): 11 was redissolved in 6 N HCl (10 mL) and refluxed overnight. The mixture was subsequently concentrated in vacuo, treated with DEAE Sephadex (acetate form), concentrated, and transferred on a Dowex WX-8 (H^+ form) column. Hydrochloric acid was first eluted with water, and the amino acid subsequently with 2 N ammonia. The eluate was evaporated to dryness, dissolved in water, and the solution was adjusted to pH 3. Ethanol was then added until crystallization began. After one night at −20 °C, the crystals were collected to give 142 mg proline.HCl, with all the characteristics of an authentic sample (60%, 0.9 mmol). $[\alpha]_D^{23}$ = −83.7 (*c* = 4, H_2O), ref. (L-proline) $[\alpha]_D^{20}$ = −84 (*c* = 4, H_2O). ^{1}H NMR (300 MHz, D_2O): δ = 2.13 (m, 3 H, 3-H, 4-H), 2.44 (m, 1 H, 3-H), 3.40 (m, 2 H, 5-H), 4.42 (dd, 1 H, 2-H) ppm. ^{13}C NMR (75.5 MHz, D_2O): δ = 24.1 (C-4), 29.1 (C-3), 47.0 (C-5), 60.4 (C-2), 172.7 (C-1) ppm.

***tert*-Butyl 2-Amino-4-cyanobutanoate (12):** The protected amino acid (434 g, 1.25 mmol) was dissolved in THF (10 mL), and a 15% citric acid solution (5 mL) was added. After stirring the mixture for 3 h, it was diluted with water (10 mL) and extracted with ether (2 × 25 mL). The water phase was then neutralized with K_2CO_3 to pH 12−13 and extracted with ethyl acetate (3 × 20 mL). The combined organic phases were dried with $MgSO_4$ and concentrated in vacuo. The mixture was further purified over a silica column (PE/ethyl acetate, 85:15) to obtain the product in 96% yield (221 mg, 1.20 mmol). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.47 (s, 9 H, *t*Bu), 1.82 (m, 1 H, 3-H), 2.09 (m, 1 H, 3-H), 2.54 (m, 2 H, 4-H), 3.39 (m, 1 H, 2-H) ppm. ^{13}C NMR (50.1 MHz, D_2O): δ = 14.19 (C-4), 28.02 (C-3), 54.03 (C-2), 84.04 (*t*Bu), 121.43 (C5), 175.34 (C1) ppm.

L-Ornithine·HCl: The purified protected nitrile (1.0 g, 5.3 mmol) was dissolved in acetic acid (10 mL), and platinum oxide (300 mg) was added. The mixture was then shaken vigorously under H_2 for 16 h. Thereafter, the mixture was filtered to remove the platinum catalyst and concentrated in vacuo. The raw product was redissolved in 6 N HCl (10 mL) and refluxed overnight. The mixture was subsequently concentrated in vacuo, treated with DEAE Sephadex (acetate form), concentrated, and transferred on a Dowex WX-8 (H^+ form) column. Hydrochloric acid was first eluted with water, and the amino acid subsequently with 2 N ammonia. The eluate was evaporated to dryness, dissolved in water, and the solution was adjusted to pH 3. Ethanol was then added until crystallization began. After one night at −20 °C, the crystals were collected to give 450 mg ornithine.HCl, with all the characteristics of an authentic sample (50%, 2.7 mmol). $[\alpha]_D^{20}$ = +20.5 (*c* = 1, 6 N HCl), ref. $[\alpha]_D^{20}$ = +22.0 (*c* = 4, 6 N HCl). NMR (50.1 MHz, D_2O): δ = 23.32 (C-4), 27.96 (C-3), 39.44 (C-5), 54.64 (C-2), 174.58 (C-1) ppm. FTIR (neat, fingerprint region): $\tilde{\nu}$ = 350.2, 397.8, 458.4, 555.7, 669.9, 757.7, 780.6, 845.6, 878.6, 935.1, 981.9, 1039.7, 1098.9, 1131.0, 1146.1, 1243.3, 1287.1, 1329.4, 1345.9, 1363.4, 1419.8, 1438.3, 1474.4 cm^{-1}.

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