Asymmetric Synthesis of [2,3-$^{13}C_2$,^{15}N]-4-Benzyloxy-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-oxazine-2-one via Lipase TL-Mediated Kinetic Resolution of Benzoin: General Procedure for the Synthesis of [2,3-$^{13}C_2$,^{15}N]-L-Alanine

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Lipase TL-mediated kinetic resolution of benzoin proceeded to give the corresponding optically pure (*R*)-benzoin (*R*)-**1**. On the other hand, (*S*)-benzoin *O*-acetate (*S*)-**7** could be hydrolyzed without epimerization to give (*S*)-benzoin (*S*)-**1** under alkaline conditions. Furthermore, both enantiomers of benzoin (**1**) were converted to [^{15}N]-(1*R*,2*S*)- and (1*S*,2*R*)- 2-amino-1,2-diphenylethanol (**3a** and **3b**), respectively, according to the procedure reported previously. [2,3-$^{13}C_2$,^{15}N]-(5*S*,6*R*)-4-benzyloxy-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-oxazine-2-one (**10**) was synthesized from ethyl [1,2-$^{13}C_2$]-bromoacetate and (1*R*,2*S*)-2-amino-1,2-diphenylethanol (**3b**) in three steps. Finally, [2,3-$^{13}C_2$,^{15}N]-L-alanine (**12**) was prepared via alkylation of the lactone **10** and hydrogenation of the alkylated product **11**.

Introduction

Amino acids[1] serve a central role in biology and chemistry. They are the fundamental constituents of proteins and chemical mediators of nitrogen metabolism and provide the raw materials from which a large number of biologically important primary and secondary metabolites are constructed.[2] Application of NMR[3] and mass spectrometric analysis to stable isotopically labeled compounds has proven useful for research on amino acid and protein metabolism.[4] In particular, the use of multiply labeled amino acids has become important as an internal standard for the study of metabolic transformation using mass spectrometric analysis.[5] In addition, multiply labeled amino acids serve as starting materials for the preparation of uniformly labeled peptides for use in multidimensional heteronuclear correlation NMR spectroscopic analyses.[6] In our own work,[7] we have extensively demonstrated that (5*S*,6*R*)- and (5*R*,6*S*)-4-CBz- and -*t*-BOC-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-ones (**4a** and **4b**) are useful as chiral, nonracemic glycine templates for the synthesis of structurally diverse α-amino acids (Scheme 1).

To synthesize oxazinones **4a** and **4b**, it is necessary to prepare *erythro*-2-amino-1,2-diphenylethanol **3a** and **3b**

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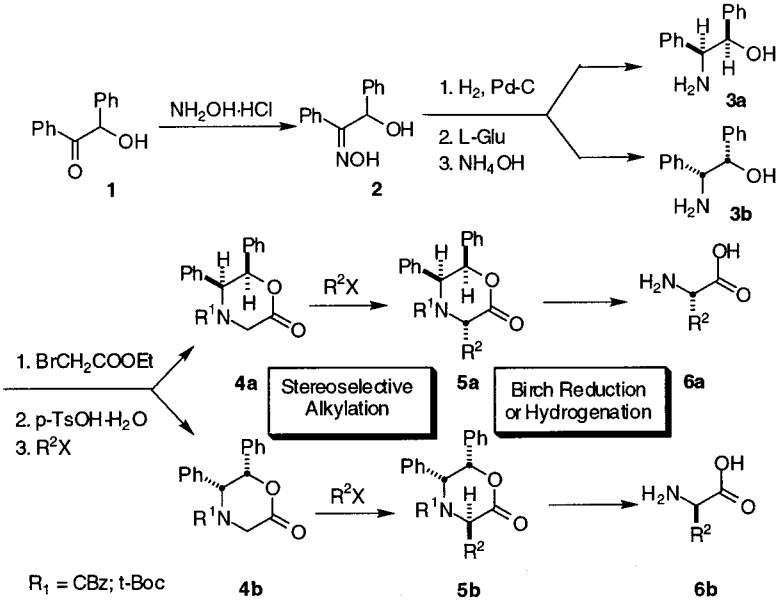
Scheme 1



in optically pure form. The preparation of the optically pure amino alcohols **3a** and **3b** has recently received widespread attention due to the use of these amino alcohols as chiral auxiliaries in asymmetric synthesis,[8] chiral stationary phase for HPLC applications,[9] and ligands in asymmetric catalysis.[10] Several methods have been described in the literature for the synthesis of both antipodes of amino alcohols **3a** and **3b**. The classical synthesis of these substances involves the resolution of racemic *erythro*-2-amino-1,2-diphenylethanol, obtained by the hydrogenation of benzoin oxime (**2**), with glutamic acid as Tishler and co-workers reported in 1951 (Scheme 1).[11] More recently, the asymmetric dihydroxylation (AD) of *trans*-stilbene followed by amination has been described by Sharpless and co-workers.[12] Fujisawa and co-workers reported the asymmetric reduction of 1,2-diaryl-2-benzyloxyiminoethanones[13] and Davis and co-workers reported an asymmetric synthesis of benzoin oxime by the asymmetric enolate oxidation of deoxybenzoin.[14] Despite the existence of these procedures, there remains a need for a practical and convenient preparation of optically pure benzoin, from which a host of important optically active compounds, including the *erythro*-2-amino-1,2-diphenylethanols, can be accessed. The Tishler procedure,[11] which involves a rather time-consuming crystallization procedure, has limited large (>10 kg) throughput. This limitation has prompted us to investigate an alternative approach. Several synthetic procedures have been reported for the preparation of pure benzoin.[14,15] Demir and co-workers reported an enzymatic synthesis of chiral benzoin in aqueous medium, but this procedure gave low yields and moderate enantiomeric ratios (er's) of benzoin.[16] Lipase-mediated kinetic resolutions of secondary alcohols in nonaqueous medium are well-known to be a powerful tool in organic synthesis to prepare chiral synthetic intermediates.[17] Adam and co-workers have reported lipase-mediated kinetic resolutions[18] of α-hydroxy ketones by using Amano PS and Amano AK, but these procedures gave low conversion and required relatively long reaction times. Despite these reports, there is relatively little known about the kinetic enzymatic resolution of benzoins. In a preliminary paper,[19] we have recently reported the efficient enzymatic optical resolution of benzoin **1** and the preparation of optically pure (1*R*,2*S*)- and (1*S*,2*R*)-2-amino-1,2-diphenylethanol. In this paper, we wish to describe the lipase TL-mediated kinetic resolution of benzoin in detail and its conversion to [2,3-$^{13}C_2$,^{15}N]-(5*S*,6*R*)-4-CBz-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**10**), which should prove to be a useful template for the synthesis of ^{15}N,^{13}C-multiply labeled optically active α-amino acids. Finally, the synthesis of [2,3-$^{13}C_2$,^{15}N]-L-alanine (**12**) was conducted to demonstrate the utility of the doubly labeled oxazinone.

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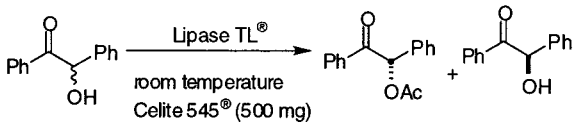
Table 1. Lipase TL-Mediated Kinetic Resolution of Benzoin



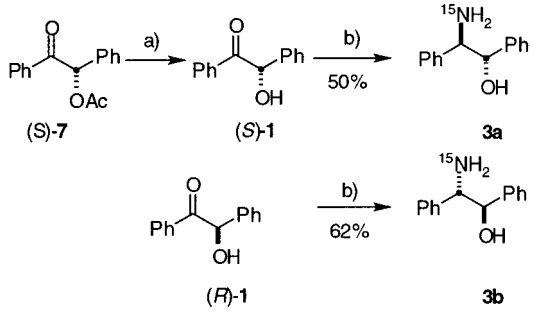
	amount (mg)					(*S*)-**7**		(*R*)-**1**	
entry	(+)-**1**	Lipase TL	solvent[a]	acyl donor[d]	reaction time (h)	yield (%)	er	yield (%)	er
1	100	10	THF[a]	VA	20	9		79	
2	100	50	THF[a]	VA	20	29		35	
3	100	100	THF[a]	VA	42	40		53	
4	100	250	THF[a]	VA	20	52		46	
5	100	100	EtOAc[a]	VA	20	2		95	
6	100	100	benzene[a]	VA	20	18		69	
7	100	100	THF[a]	IA	43	3		88	
8[e]	500	1250	THF[b]	VA	20	42	>99:1	40	97:3
9[f]	500	1250	THF[b]	VA	20	46	>99:1		
10[g]	3760	9400	THF[c]	VA	20	50	>99:1	48	96:4

[a] 5 mL of solvent was used. [b] 25 mL of solvent was used. [c] 188 mL of solvent was used. [d] VA, vinyl acetate; IA, isopropenyl acetate; 12 equiv of the acyl donor was employed. [e] 2.5 g of Celite 545 was used. [f] Reaction in the absence of Celite. [g] The enantiomeric ratios (er's) were determined by means of HPLC with a Chiralcel OD column.

Results and Discussion

Initially, we examined the kinetic resolution of (±)-benzoin (**1**) in the presence of various commercially available lipases, including PPL, Amano PS, Amano I, Amano II, and Lipase MY, UL, TL, SC, AL, OF, and QL in a mixture of vinyl acetate and THF at room temperature. Among these lipases, Lipase TL[20] was found to be the most effective (conversion rate up to 50%). Next, the reaction parameters were varied including the acyl donor, reaction time, and solvent; the results are shown in Table 1. The reaction did not proceed in hexane as a solvent. The best results were obtained when the reaction was conducted in a mixture of THF as a solvent and vinyl acetate as an acyl donor at room temperature (Table 1, entry 4). The products were easily separated by flash column chromatography on silica gel. On the basis of these results, the conditions employed in entry 4 were scaled up to 500 mg and 4000 mg of racemic benzoin, in both the presence and absence of Celite. The best results obtained (Table 1, entries 9 and 10) gave (*R*)-**1** in 41∼48% yield and 96:4 er plus (*S*)-**7** in 46∼47% yield and >99:1 er. The enantiomeric ratios of benzoin acetate and the recovered benzoin were determined by means of chiral HPLC analysis (Chiralcel OD). When the kinetic resolution of benzoin was conducted in the presence of recovered lipase TL, the exact same results were obtained and thus, although a 2.5 gm/gm excess of enzyme was found to be optimal, the enzyme was easily recovered and reused without loss of enzymatic activity. Hydrolyis[21] of the optically pure benzoin *O*-acetate (*S*)-**7** proceeded at 0 °C under alkaline conditions to give the corresponding benzoin (*S*)-**1** in 91% yield on 100 mg scale by means of flash column chromatography (Scheme 2). On the other hand, on a 1 gram scale, separation was conducted without column chromatography to give (*S*)-**7** in 76% yield. The er's of the products were >98:2.

Scheme 2[a]



[a] Reagents and conditions: (a) K_2CO_3, MeOH, H_2O, 0 °C, 91%; (b) (i) $^{15}NH_2OH-HCl$, NaOAc, EtOH, H_2O, quant; (ii) 5% Pd−C, H_2 (3 atm), 3% HCl in EtOH.

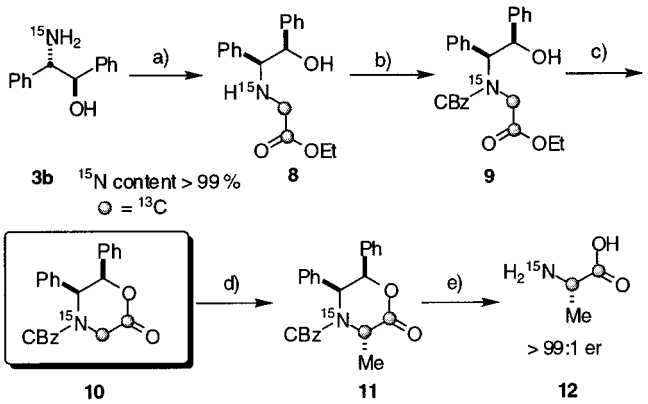
With both enantiomers of approximately optically pure benzoin in hand, we examined the preparation of the amino alcohols. Namely, the condensation of the chiral benzoins (*R*)-**1** and (*S*)-**1** with [^{15}N]-NH_2OH·HCl in a refluxing mixture of water and ethanol gave a mixture of the *E*- and *Z*-isomers of the corresponding oxime (ca. 53:47 on the basis of ^{1}H NMR analysis), which were used directly as an *E*/*Z* mixture.[11] Without further purification of the oximes, hydrogenation was performed in ethanol containing 3% HCl under an atmosphere of hydrogen (3 atm) to give the *erythro*-amino alcohols **3a** and **3b** in 50% and 62% yields, respectively (Scheme 2). The enantiomeric ratios were determined to be 99:1 to 99:2 by means of chiral HPLC analysis with a Chiral CD-Ph column. Mass spectral analysis showed that these compounds contained 99% of ^{15}N.

Next, following our published procedure,[7] transformation of the chiral ^{15}N-labeled amino alcohol **3b** to [2,3-$^{13}C_2$,^{15}N]-(5*S*,6*R*)-4-CBz-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**10**) was conducted as shown in Scheme 3. Namely, the reaction of compound **3b** with ethyl [1,2-$^{13}C_2$]bromoacetate proceeded to give compound **8**, which was employed in the next reaction without further purification. Crude **8** was easily transformed into the corresponding CBz derivative **9**. In the presence of *p*-TsOH, the lactonization of **9** with azeotropic distillation of benzene was conducted to give the lactone **10** in 62% overall yield for the three steps. To demonstrate the

(20) This lipase can be purchased from Meito Sangyo Co. Ltd (Tokyo, Japan).

(21) There are some papers that describe the hydrolysis of benzoin *O*-acetate. (a) In acidic medium: Harada, K.; Shiono, S. *Bull. Chem. Soc. Jpn.* **1984**, 57, 1040−1045. (b) Using scandium triflate: Kajiro, H.; Mitamura, S.; Mori, A.; Hiyama, T. *Tetrahedron Lett.* **1999**, *40*, 1689−1692. Kajiro, H.; Mitamura, S.; Mori, A.; Hiyama, T. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1553−1560.

Scheme 3[a]



[a] Reagents and conditions: (a) $Br^{13}CH_2{}^{13}COOEt$, Et_3N, THF; (b) CBzCl, $NaHCO_3$, $CHCl_3$; (c) *p*-TsOH, benzene; (three steps overall yield 63%); (d) $NaN(TMS)_2$, MeI, THF, 91%; (e) $PdCl_2$, H_2 (3 atm), 84%.

potential utility of the doubly labeled oxazinone thus obtained, [^{15}N,$^{13}C_2$]-labeled L-alanine was prepared. Alkylation of the lactone **10** with methyl iodide in the presence of sodium bis(trimethylsilyl)amide at −78 °C gave a single diastereomer (**11**) in 82% yield. Hydrogenation of **11** with palladium chloride in a mixture of tetrahydrofuran and ethanol under a hydrogen atmosphere (3 atm) gave the corresponding [^{15}N,$^{13}C_2$]-L-alanine **12**.[6] The er of amino acid **12** was determined to be higher than 99:1 by means of ^{1}H and ^{19}F NMR analysis of the corresponding Mosher amide.

In summary, the synthesis of [2,3-$^{13}C_2$,^{15}N]-(5*S*,6*R*)-4-CBz-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**10**) has been described. The requisite amino alcohols **3** have been obtained in high optical purity via the lipase TL-mediated kinetic resolution of benzoin, which are both commercially available starting materials. The kinetic resolution of benzoin **1** using lipase TL proceeded to give (*S*)-benzoin-*O*-acetate (*S*)-**7** and (*R*)-benzoin (*R*)-**1** in good yields. Hydrolysis of the acetate (*S*)-**7** gave the corresponding (*R*)-benzoin **1**. Both enantiomers were converted to *erythro*-amino alcohols **3a** and **b** in moderate yields. Following our previously reported procedure, **3b** was converted to optically pure [2,3-$^{13}C_2$,^{15}N]-(5*S*,6*R*)-4-CBz-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**10**), which was employed in the synthesis of [1,2-$^{13}C_2$,^{15}N]-L-alanine **12**. This protocol should prove to be general for the synthesis of multiply labeled amino acids. Our laboratory has published extensively on the enolate alkylation reactions and electrophilic substitutions of **10**, which amply demonstrates the general utility of this simple glycine template for the synthesis of a wide array of isotopically labeled amino acids and peptide isoteres.[7] The single example of the synthesis of labeled alanine reported herein, serves only to illustrate the synthetic potential of the isotopically labeled glycine templates **10**. Additional applications of these labeled substrates shall be reported on in due course from these laboratories.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto micromelting point apparatus and are uncorrected. NMR spectra were obtained on Brucker DPX-400 and DRX-500 spectrometers. IR spectra were recorded on a Jasco FT/IR-620 spectrometer. Mass spectra were determined on a Fisons VG Auto Spec instrument. High-performance liquid column chromatography (HPLC) was conducted using a JASCO UV-970 UV detector, a JASCO PU-980 pump, and a JASCO 807-IT integrator. The chiral columns Chiralcel OD and Chiral CD-Ph were purchased from Daicel Co. Ltd., Tokyo, and Shiseido Co. Ltd., Tokyo, respectively. Medium-pressure column chromatography (MPLC) was conducted using a UVILOG 5III spectrometer as the UV detector (Oyo Bunko Kiki Co., Ltd., Tokyo) and Kieselgel 60 (Merck AG, Darmstadt) as the packing material. Lipase TL was purchased from Meito Sangyo Co., Ltd., Japan. The lipase must be dried at room temperature under reduced pressure (1−2 mmHg) for 24 h before use. All other reagents were employed in the reaction without further purification. ^{15}N-Hydroxylamine hydrochloride (catalog no. T85-70214, min 99 atom %) and 1,2-$^{13}C_2$-ethyl bromoacetate (catalog no. T83−02517, min 99 atom %) were purchased from ISOTEC Co. Ltd. (USA).

Kinetic Resolution of Benzoin 1. (a) Column Separation. A mixture of benzoin **1** (100 mg, 0.47 mmol), THF (5 mL), Lipase TL (250 mg), and vinyl acetate (0.5 mL, 5.64 mmol) was stirred at room temperature for 15 h. After filtration, the filtrate was concentrated in vacuo to give an oily residue. The residue was purified with MPLC (hexane−AcOEt solvent system) to give (*S*)-acetate **2** [[α]$_D$ +217° (*c* = 0.35, $CHCl_3$) (lit.[21a] (*R*)-benzoin acetate [α]$_D$ −214.3° (*c* = 1.0, $CHCl_3$))] and recovered (*R*)-benzoin **1**. [[α]$_D$ −118° (*c* = 0.2, acetone) (lit.[21a] [α]$_D$ −111.6° (*c* = 1.0, acetone))]; physical data for this compound were identical with those reported in the literature.[21] The er's were determined by means of HPLC analysis with a chiral column, as described below.

(b) Using Recycled Lipase TL: Semi-Large Scale. ***The lipase must be dried at room temperature for 24 h under reduced pressure before use.*** A mixture of benzoin **1** (3.76 g, 17.7 mmol), THF (188 mL), Lipase TL (9.40 g), and vinyl acetate (19.6 mL, 213 mmol) was stirred at room temperature for 20 h. After filtration, the filtrate was concentrated in vacuo to give a pale yellow residue (5.3 g). *i*-Pr_2O (100 mL) was then added to the residue, which was then heated at 40 °C for several minutes and filtered to give optically pure (*R*)-benzoin **1** (0.62 g, 17%). The filtrate was evaporated under reduced pressure to give a residue. A second trituration with *i*-Pr_2O (80 mL) gave more optically pure (*R*)-benzoin **1** (0.45 g, 12%). Finally, the filtrate was evaporated and purified with MPLC (hexane−AcOEt solvent system) to give the (*S*)-acetate (*S*)-**7** (2.26 g, 50%) as a less polar component and recovered (*R*)-benzoin (*R*)-**1** (1.0 g, 27%). As a result, total recovered (*R*)-**1** was 1.80 g (48%).

Determination of Enatiomeric Ratio (er) of Recovered Benzoin 1 and Benzoin *O*-Acetate 7 by Means of HPLC Analysis with a Chiral Column. Column: Daicel CHIRALCEL OD column (4.6 × 250 mm). Solvent: hexane/^{i}PrOH = 9:1. UV wavelength: 270 nm. Flow rate: 1.0 mL/min. Pressure: 20 kg/cm^2. (*R*)-benzoin **1**; t_R = 17.4 min. (*S*)-benzoin (*S*)-**1**; t_R = 12.2 min. (*R*)-benzoin *O*-acetate (*R*)-**7**; t_R= 6.5 min. (*S*)-benzoin-*O*-acetate (*S*)-**7**; t_R= 10.0 min.

Hydrolysis of (*S*)-Benzoin Acetate (*S*)-7. A mixture of (*S*)-acetate 2 (100 mg, 0.39 mmol), MeOH (30 mL), H_2O (30 mL), and K_2CO_3 (54 mg, 0.39 mmol) was stirred at room temperature for 10 min. After H_2O (150 mL) was added to the reaction mixture, the aqueous solution was extracted with AcOEt (50 mL × 3). The organic layer was washed with saturated aqueous NaCl (50 mL × 3), dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give a residue, which was purified with flash column chromatography (hexane−AcOEt = 6:1) to give (*S*)-benzoin (*S*)-**1** (91%) as a colorless solid: [[α]$_D$ +120° (*c* = 0.2, acetone) ((*R*)-benzoin lit.[21a] [α]$_D$ −111.6° (*c* = 1.0, acetone))]. Physical data for this compound were identical with those reported in the literature.[21] The er of the product was determined by means of the HPLC method mentioned above.

Preparation of ^{15}N-(1*R*,2*S*)-*erythro*-2-Amino-1,2-diphenylethanol 3b from (*R*)-Benzoin 1. A mixture of (*R*)-benzoin **1** (1.0 g, 4.7 mmol), NaOAc (0.95 g, 11.6 mmol), $^{15}NH_2OH{\cdot}HCl$ (0.8 g, 11.5 mmol), EtOH (40 mL), and H_2O (8 mL) was refluxed for 2 h. After evaporation in vacuo, Et_2O

(100 mL) was added to the residue. The organic phase was washed with saturated aqueous $NaHCO_3$ (40 mL × 3), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure to give a residue that was a mixture of *E* and *Z* oxime (53:47). Without further separation, the mixture was employed in the hydrogenation. A mixture of the oxime (1.0 g, 2.11 mmol), 5% Pd−C (0.117 g), and 3% HCl in EtOH (21 mL) was hydrogenated under 3 kg/cm^2 of H_2 for 6 h. After the reaction, H_2O (100 mL) was added and the palladium catalyst was filtered off through Celite 545. The pH of the aqueous solution was made alkaline through addition of concentrated aqueous NH_3, which caused the appearance of a crystalline precipitate. After filtration and drying under reduced pressure, compound **3b** (0.61 g, 62%) was obtained as a colorless solid. The threo isomer could not be detected in the ^{1}H NMR spectrum. The er of the product was determined by means of HPLC analysis with a chiral column as described below: mp 142−144 °C; $[\alpha]_D$ −7.6° (*c* = 0.6, EtOH); ^{1}H NMR (400 MHz, 300 K, $CDCl_3$) δ 7.33−7.21 (m, 10H), 4.75 (d, 1H, *J* = 6.2 Hz), 4.17 (d, 1H, *J* = 6.2 Hz), 1.54 (brs, 3H); ^{13}C NMR (100 MHz, 300 K, $CDCl_3$) δ 141.5, 140.7, 128.3, 128.1, 127.8, 127.6, 127.6, 126.9, 78.4 (d, *J* = 3.1 Hz), 61.9 (d, *J* = 4.7 Hz); ^{15}N NMR (50 MHz, 300 K, CD_3OD, CH_3NO_2 as internal standard) δ −352.16 ppm; IR (film) 3324, 3272 cm^{-1} (NH_2, OH); HRMS (FAB) calcd for $C_{14}H_{16}{}^{15}NO$ 215.1202 (M^+ + H), found 215.1202; er 98:2.

Preparation of ^{15}N-(1*S*,2*R*)-*erythro*-2-Amino-1,2-diphenylethanol 3a from (*S*)-Benzoin 1. Compound **3a** was prepared from (*S*)-**1** (0.46 g, 2.19 mmol) in two steps, in a manner similar to that for **3b**: yield 0.22 g (50%); mp 142−144 °C; $[\alpha]_D$ +8.3° (*c* = 0.6, EtOH); ^{1}H NMR (400 MHz, 300 K, $CDCl_3$) δ 7.33−7.20 (m, 10 H), 4.74 (d, 1H, *J* = 6.3 Hz), 4.16 (d, 1H, *J* = 6.3 Hz), 1.72 (brs, 3H); ^{13}C NMR (100 MHz, 300 K, $CDCl_3$) δ 141.5, 140.7, 128.2, 128.1, 127.7 (overlap), 127.5, 126.9, 78.3 (*d*, *J* = 3.0 Hz), 61.8 (*d*, *J* = 4.4 Hz); ^{15}N NMR (50 MHz, CD_3OD, CH_3NO_2 as a internal standard) δ −352.08 ppm; IR (film) 3324, 3271 cm^{-1} (NH_2, OH); HRMS (FAB) calcd for $C_{14}H_{16}{}^{15}NO$ 215.1202 (M^+ + H)., found 215.1201; er 98:2.

Determination of Enatiomeric Ratio (er) of Amino Alcohols 3a and 3b by Means of HPLC Analysis with a Chiral CD-Ph Column. Column: Shiseido Chiral CD-Ph column (4.6 × 250 mm). Solvent: MeCN/0.5 M $NaClO_4$ = 6:4. UV wavelength: 254 nm. Flow rate: 0.4 mL/min. Pressure: 32 kg/cm^2. (2*S*,3*R*)-**3b**; t_R = 13.3 min. (2*R*,3*S*)-**3a**; t_R = 17.2 min.

Preparation of [2,3-$^{13}C_2$,^{15}N]-(5*S*,6*R*)-4-CBz-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (10). To a mixture of **3b** (0.85 g, 3.99 mmol) and 1,2-$^{13}C_2$-ethyl bromoacetate (1.0 g, 5.99 mmol) and dry THF solution (20 mL) was added Et_3N (1.1 mL, 8.0 mmol) at 0 °C under an Ar atmosphere. The resulting mixture was stirred at room temperature for 18 h. The mixture was filtered to remove Et_3N·HBr. The filtrate was evaporated under reduced pressure to remove excess Et_3N, THF, and 1,2-$^{13}C_2$-ethyl bromoacetate to give crude **8** as a solid (1.29 g), which was employed to the next reaction without further purification.

To a mixture of crude **8**, CH_2Cl_2 (20 mL), and saturated $NaHCO_3$ (20 mL) was added benzyl chloroformate (0.82 g, 4.8 mmol) at 0 °C under an Ar atmosphere. After the resulting mixture was stirred vigorously at room temperature for 15 h, the aqueous layer was separated and extracted with CH_2Cl_2 (20 mL × 3), and the combined organic phases were washed with H_2O (30 mL × 3), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure to give crude **9** as a colorless oil. To a stirred solution in benzene (200 mL) of the crude *N*-CBz ethyl ester **9** obtained above in a 500 mL one-neck round-bottom flask equipped with a Soxhlet extractor packed with 60 g of $CaCl_2$ was added *p*-TsOH·H_2O (0.07 g, 0.37 mmol). The mixture was brought to reflux for 8 h. The solvent was evaporated under reduced pressure to give a solid. After addition of CH_2Cl_2 (50 mL) to the residue, the resulting solution was washed with 5% $NaHCO_3$ (20 mL × 1) and H_2O (20 mL × 2). The solvent was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure to give a residue, which was recrystallized from EtOH to give [2,3-$^{13}C_2$,^{15}N]-(5*S*,6*R*)-4-CBz-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one **10** as colorless needles (0.98 g, 63%): mp 211−213 °C; $[\alpha]_D$ −70.4° (*c* = 0.14, CH_2Cl_2); ^{1}H NMR (400 MHz, 383 K, DMSO-d_6) δ 7.28−7.26 (m, 6H), 7.21−7.08 (m, 7H), 6.73 (d, 2H, *J* = 7.3 Hz), 6.21 (brs, 1H), 5.32 (brs, 1H), 5.11 (d, 1H, *J* = 12.7), 5.06 (d, 1H, *J* = 12.7), 4.64 (ddd, 1H, *J* = 143, 17.7, 6.5 Hz), 4.57 (ddd, 1H, *J* = 143, 17.7, 6.5 Hz); ^{13}C NMR (100 MHz, 300 K, $CDCl_3$) δ 167.2 (d, *J* = 56 Hz), 166.8 (d, *J* = 56), 45.3 (dd, *J* = 56, 11 Hz), 61.9 (d, *J* = 4.7 Hz); ^{15}N NMR (50 MHz, 300 K, CD_3OD, CH_3NO_2 as internal standard) δ ppm; IR (film) 1705 cm^{-1} (C═O); HRMS (FAB) calcd for $C_{22}{}^{13}C_2H_{22}{}^{15}NO_4$ (M^+ + H) 391.1586, found 391.1588. Anal. Calcd for $C_{22}{}^{13}C_2H_{21}{}^{15}NO_4$: C, 74.33; H, 5.42; N, 3.84. Found: C, 73.95; H, 5.48; N, 3.65.

Methylation of [2,3-$^{13}C_2$,^{15}N]-(5*S*,6*R*)-4-CBz-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one 10. To a solution of compound **10** (0.31 g, 0.81 mmol), MeI (0.5 mL, 8.1 mmol), and dry THF (25 mL) was added 1.0 M $NaN(TMS)_2$ in THF (1.2 mL, 1.2 mmol) dropwise at −78 °C under an Ar atmosphere. After the resulting solution was stirred at the same temperature for 30 min, the reaction mixture was poured into EtOAc (200 mL). The organic layer was washed with H_2O (100 mL × 2) and saturated aqueous NaCl (100 mL × 1), dried over anhydrous $MgSO_4$, filtered, concentrated, and purified by means of silica gel flash chromatography (eluted with $CHCl_3$/EtOAc = 25:1) to give compound **11** as a colorless solid (0.30 g, 91% yield): mp 188−190 °C; $[\alpha]_D$ −49.8° (*c* = 0.47, CH_2Cl_2); ^{1}H NMR (400 MHz, 383 K, d_6-DMSO) δ 7.29−7.22 (m, 6H), 7.20−7.15 (m, 1H), 7.12−7.08 (m, 6H), 6.61 (d, 1H, *J* = 7.2 Hz), 6.21 (d, 1H, *J* = 2.8 Hz), 5.31 (brs, 1H), 4.95 (dm, 1H, *J* = 150), 5.07 (d, 1H, *J* = 12.7), 5.00 (d, 1H, *J* = 12.7 Hz), 4.95 (dm, 1H, *J* = 150), 1.80−1.75 (m, 3H); ^{13}C NMR (100 MHz, 300 K, $CDCl_3$) δ 170.1 (d, *J* = 56 Hz), 170.0 (d, *J* = 55 Hz), 52.9 (dd, *J* = 55, 9 Hz), 52.8 (dd, *J* = 56, 11 Hz); ^{15}N NMR (50 MHz, 300 K, CD_3OD, CH_3NO_2 as internal standard) δ −283.98 (d, *J* = 9.0 Hz), −284.46 (d, *J* = 11 Hz) ppm; IR (film) 1709 (C═O) cm^{-1}; FAB-MS 405 (M^+ + H), 361 (M^+ − CO_2H); HRMS (FAB) calcd for $C_{23}{}^{13}C_2H_{24}{}^{15}NO_4$ (M^+ + H) 405.174278, found 405.174780.

Hydrogenation of Compound 11. Preparation of [1,2-$^{13}C_2$,^{15}N]-L-Alanine 12. A mixture of lactone **11** (0.20 g, 0.56 mmol), $PdCl_2$ (0.025 g), EtOH (5 mL), and THF (5 mL) was stirred at room temperature under H_2 (3 kg/cm^2) for 4 days. After filtration through Celite 545, the filtrate was diluted with H_2O (30 mL). The solvent was washed with Et_2O (15 mL × 2) and evaporated in vacuo to a small volume (2 mL). After the solution was adjusted to pH 2−3 by adding 5% HCl, the solution was loaded onto a Dowex 50WX4-100 ion-exchange resin and eluted with 0.5 N NH_4OH. The elutant was lyophilized to give L-alanine as a colorless solid (0.038 g, 89%): mp 290−295 °C (EtOH−H_2O); $[\alpha]_D$ +2.5° (*c* = 0.41, H_2O); ^{1}H NMR (400 MHz, 300 K, D_2O) δ 3.83 (ddq, 1H, *J* = 145, 7.2, 5.2 Hz), 1.55−1.51 (m, 3H); ^{13}C NMR (100 MHz, 300 K, D_2O) δ 175.8 (d, *J* = 54 Hz), 50.5 (dd, *J* = 54, 5.5 Hz); ^{15}N NMR (50 MHz, 300 K, D_2O, CH_3NO_2 as a external standard) δ −337.24 (d, *J* = 5.5 Hz) ppm; FAB-MS 93 (M^+ + H); HRMS (FAB) calcd for $^{12}C^{13}C_2H_8{}^{15}NO_2$ 93.059248, found 93.059217.

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