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TETRAHEDRON

An Asymmetric Synthesis of L-[3-^{13}C]Phenylalanine and L-[3-^{13}C]Tyrosine from [^{13}C]Carbon Monoxide[†]

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Abstract: L-[3-^{13}C]Phenylalanine and L-[3-^{13}C]tyrosine were synthesized. [α-^{13}C]Benzyl bromides were prepared from [^{13}C]carbon monoxide *via* the palladium-catalyzed carboalkoxylation of aryl halides. The asymmetric carbon corresponding to the 2-position in phenylalanine was introduced by the diastereoselective alkylation of Dellaria's oxazinone with [α-^{13}C]benzyl bromides. Finally, ethanolysis, deprotection, hydrogenolysis and acid hydrolysis of the resulting alkylated oxazinones gave L-[3-^{13}C]phenylalanine and L-[3-^{13}C]tyrosine in high optical purity.

INTRODUCTION

Stable isotope-labeled amino acids are useful for biological studies of metabolism, the diagnosis of disease, biosynthetic studies of natural products and the structure analysis of peptides and proteins.[1] Many methods are available for synthesizing optically active amino acids, including asymmetric amination, enantioselective alkylation of glycine equivalents, asymmetric Strecker reaction, asymmetric hydrogenation of dehydroamino acids and enzymatic synthesis.[2] The use of chiral auxiliaries and chiral glycine equivalents, such as Evans' oxazolidinones, Oppolzer's sultams, Williams' oxazinones, Schöllkopf's bis-lactim ethers and Seebach's imidazolidinones, is convenient for the ^{13}C-labeling of optically active amino acids.[3] Dellaria and Santarsiero reported the oxazinone **1** as a chiral glycine equivalent for the synthesis of optically active amino acids in 1989.[4] In this paper, we describe the asymmetric synthesis of L-[3-^{13}C]phenylalanine (**2**) and L-[3-^{13}C]tyrosine (**3**) from [^{13}C]carbon monoxide and Dellaria's oxazinone **1**.

[†] This paper is dedicated to Professor A. I. Scott on the occasion of his 70th birthday.

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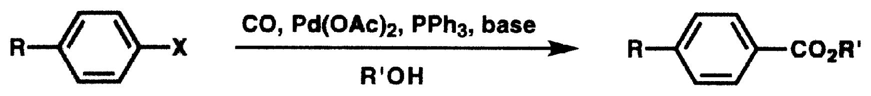
RESULTS AND DISCUSSION

Investigation of Carboalkoxylation

We planned to synthesize L-[3-^{13}C]phenylalanine (**2**) and L-[3-^{13}C]tyrosine (**3**) from Dellaria's oxazinone **1** and [^{13}C]alkyl halides, [α-^{13}C]benzyl bromide (**4**) and 1-bromo[^{13}C]methyl-4-(methoxymethyloxy)benzene (**5**). The [^{13}C]alkyl halides were prepared from [^{13}C]carbon monoxide by using the palladium-catalyzed carboalkoxylation of aryl halides.[5]

The carboalkoxylation was optimized with unlabeled carbon monoxide (Table 1). The carboalkoxylation of bromobenzene (**6a**) with palladium diacetate, triphenylphosphine and diisopropylethylamine in butanol under atmospheric pressure of carbon monoxide proceeded smoothly to afford butyl benzoate (**7a**) in 72% yield (entry 1). However, at higher pressure of carbon monoxide in methanol, the resulting methyl benzoate (**7b**) was obtained in low yield (entry 2). The use of iodobenzene (**6b**) as the starting aryl halide afforded biphenyl as the major product in 70% yield, while the yield of the desired **7a** was only 3% (entry 3). On the other hand, the reaction of 1-bromo-4-(methoxymethyloxy)benzene (**8a**), prepared by treatment of 4-bromophenol with NaH and chloromethyl methyl ether, under atmospheric pressure of carbon monoxide afforded the corresponding butyl benzoate (**9a**) in 42% yield, with recovery of the starting material **8a** in 44% yield (entry 4). However, the reaction of 1-iodo-4-(methoxymethyloxy)benzene (**8b**) proceeded at 8 kgf/cm^2 pressure of carbon monoxide to afford methyl 4-(methoxymethyloxy)benzoate (**9b**) in excellent yield (entry 6). These conditions were used for the synthesis of the ^{13}C-labeled compounds.



6a: R = H, X = Br
6b: R = H, X = I
8a: R = OMOM, X = Br
8b: R = OMOM, X = I

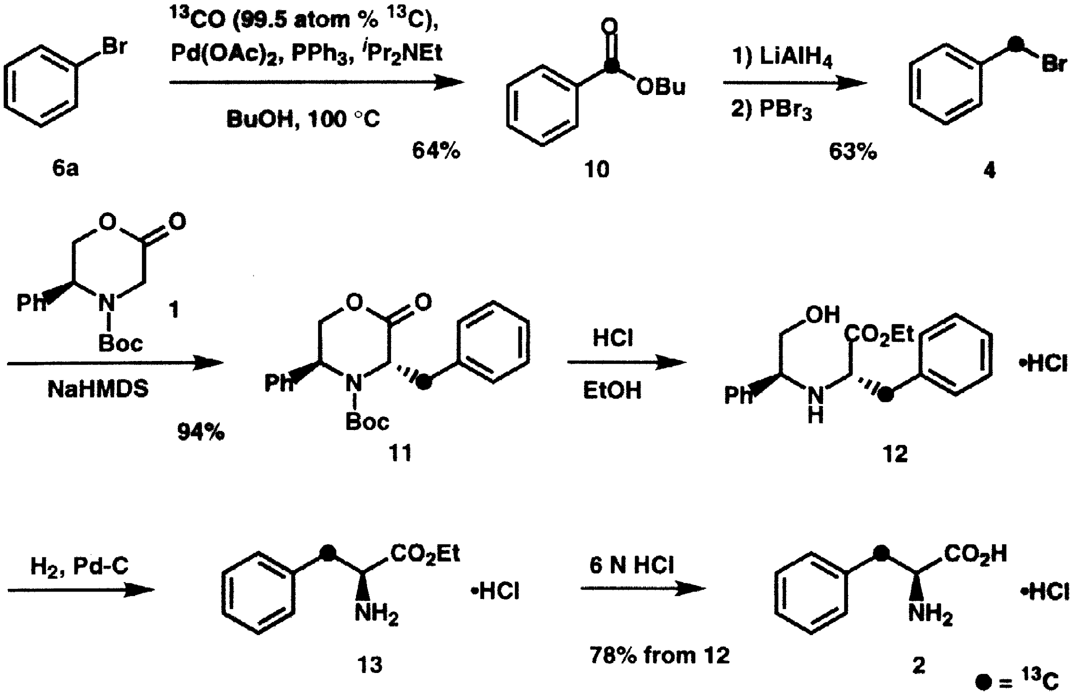
7a: R = H, R' = Bu
7b: R = H, R' = Me
9a: R = OMOM, R' = Bu
9b: R = OMOM, R' = Me

entry	substrate	temperature	pressure	R'	base	time	yield
1	6a	100 °C	atmospheric	Bu	^{i}Pr$_2$NEt	24 h	7a, 72%
2	6a	120 °C	5 kgf/cm^2	Me	Et$_3$N	12 h	7b, 43%
3	6b	100 °C	atmospheric	Bu	^{i}Pr$_2$NEt	24 h	7a, 3%
4	8a	100 °C	atmospheric	Bu	^{i}Pr$_2$NEt	24 h	9a, 42%
5	8b	100 °C	atmospheric	Bu	^{i}Pr$_2$NEt	24 h	many products
6	8b	120 °C	8 kgf/cm^2	Me	Et$_3$N	5 h	9b, 99%

Table 1

Synthesis of L-[3-^{13}C]Phenylalanine (2)

Synthesis of L-[3-^{13}C]phenylalanine (**2**) was performed as follows (Scheme 1). The carboalkoxylation of bromobenzene (**6a**) with [^{13}C]carbon monoxide (99.5 atom % ^{13}C) under the above conditions gave butyl [*carbonyl*-^{13}C]benzoate (**10**) in 64% yield. Reduction of **10** with $LiAlH_4$ followed by treatment of the resulting [α-^{13}C]benzyl alcohol with phosphorus tribromide gave [α-^{13}C]benzyl bromide (**4**) in 63% yield. [α-^{13}C]Benzyl bromide (**4**) was also prepared from [*carbonyl*-^{13}C]benzoic acid (99 atom % ^{13}C) by reduction with $LiAlH_4$ followed by treatment with phosphorus tribromide.

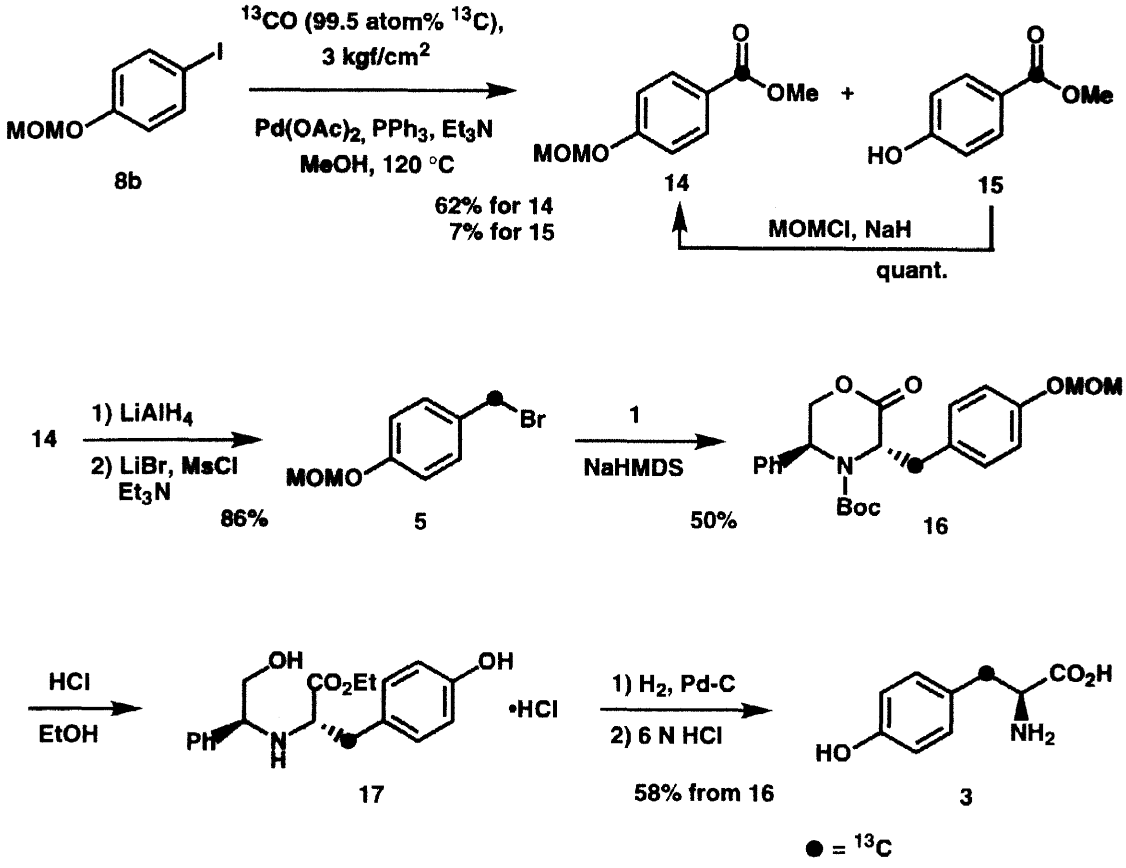


Scheme 1

The next step is the key reaction in the synthesis, introducing a chiral center corresponding to the 2-position in the amino acid into the oxazinone **1** by means of the alkylation with **4**. The oxazinone **1** was prepared from (*S*)-2-phenylglycinol and phenyl bromoacetate.[3] The alkylation of the oxazinone **1** was achieved *via* the enolate, prepared by treatment of **1** with sodium bis(trimethylsilyl)amide (NaHMDS) in THF-DME at -78 °C, by addition of [α-^{13}C]benzyl bromide (**4**) at that temperature to give the phenyl[^{13}C]methyloxazinone **11** in 94% yield from **4**. The other diastereomer of **11**, which was separated by crystallization, was also obtained in 0.5% yield. Thus, the reaction proceeded with high diastereoselectivity. Solvolysis and removal of the Boc group of **11** with HCl-ethanol, and cleavage of the C-N bond at the benzyl position of the resulting **12** by hydrogenolysis on Pd-C gave L-[3-^{13}C]phenylalanine ethyl ester hydrochloride (**13**). Finally, acid hydrolysis of **13** gave L-[3-^{13}C]phenylalanine hydrochloride (**2**) in 78% yield from the phenyl[^{13}C]methyloxazinone **11**. The chemical structure of **2** was confirmed by EI-MS, ^{1}H-NMR, ^{13}C-NMR and IR analyses. The ^{13}C-NMR spectrum of **2** showed the enriched peak at 38.4 ppm corresponding to the 3-position of phenylalanine. The optical purity was over 99% ee and the absolute configuration was L, as determined by HPLC-CD analysis using a chiral column.[6]

Synthesis of L-*[3-^{13}C]Tyrosine (3)*

Synthesis of L-[3-^{13}C]tyrosine (**3**) was accomplished by the same procedure as employed for the synthesis of L-[3-^{13}C]phenylalanine (**2**) (Scheme 2). The palladium-catalyzed carboalkoxylation of 1-iodo-4-(methoxymethyloxy)benzene (**8b**) was performed in methanol at 3 kgf/cm^2 of [^{13}C]carbon monoxide, which was the maximum pressure possible in the bomb, to afford methyl 4-(methoxymethyloxy)[*carbonyl*-^{13}C]benzoate (**14**) and methyl 4-hydroxy[*carbonyl*-^{13}C]benzoate (**15**) in 62% and 7% yields, respectively. The deprotected product **15** was easily converted to the MOM ether **14** with NaH and chloromethyl methyl ether in quantitative yield. Reduction of **14** with $LiAlH_4$ and bromine displacement of the resulting hydroxyl group with LiBr, triethylamine and mesyl chloride gave 1-bromo[^{13}C]methyl-4-(methoxymethyloxy)benzene (**5**) in 86% yield. As expected, alkylation of the oxazinone **1** with **5** proceeded with high diastereoselectivity to give the alkylated oxazinone **16** in 50% yield from **5**. In the same manner as in the L-[3-^{13}C]phenylalanine synthesis, the alkylated oxazinone **16** was converted to L-[3-^{13}C]tyrosine (**3**) *via* **17** in 58% yield and over 99% ee, as determined by HPLC-CD analysis.



Scheme 2

Thus, asymmetric and selective syntheses of L-[3-^{13}C]phenylalanine (**2**) and L-[3-^{13}C]tyrosine (**3**) were achieved by using Dellaria's chiral oxazinone **1** and [α-^{13}C]benzyl bromides labeled from [^{13}C]carbon monoxide, *via* palladium-catalyzed carboalkoxylation.

EXPERIMENTAL

Materials

[^{13}C]Carbon monoxide (99.5 atom % ^{13}C) was purchased from ISOTEC, Inc. [*carbonyl*-^{13}C]Benzoic acid (99 atom % ^{13}C) was purchased from ICON.

Instruments

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-GSX 400 or a Varian Gemini 300/2000 Fourier-transform spectrometer. The chemical shifts are reported in δ values relative to tetramethylsilane (TMS) at 0 ppm in $CDCl_3$ or HOD at 4.7 ppm in D_2O for ^{1}H-NMR and $CDCl_3$ at 77.0 ppm or 3-(trimethylsilyl)propionic-*2,2,3,3,-d$_4$* acid sodium salt (TSP) at 0 ppm in D_2O for ^{13}C-NMR. IR spectra were recorded on a JASCO VALOR-III Fourier-transform spectrometer. EI-MS were obtained with a JEOL JMS-DX-302 double-focusing spectrometer. HPLC-CD analyses were carried out on a JASCO 800 Series HPLC system with a JASCO J-720 CD spectrophotometer as a detector. The column was Crown Pak CR(-) (150 mm x 4 mm i.d.), purchased from Daicel. Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected.

Butyl [carbonyl-^{13}C]Benzoate (10)

To a stirred solution of palladium acetate (56 mg, 0.25 mmol) and triphenylphosphine (0.26 g, 1.0 mmol) in butanol (1.9 ml, 1.57 g, 21.2 mmol) were added *N,N*-diisopropylethylamine (3.31 ml, 2.46 g, 19.0 mmol) and bromobenzene (**6a**) (1.8 ml, 2.70 g, 17.2 mmol) at room temperature. The mixture was heated and stirred under a [^{13}C]carbon monoxide (99.5 atom % ^{13}C) atmosphere at 100 °C for 49 h. The reaction mixture was partitioned between ether and water. The aqueous layer was extracted three times with ether. The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated. The resulting brown oil was purified by distillation under reduced pressure to give butyl [*carbonyl*-^{13}C]benzoate (**10**) (1.96 g, 64%) as a colorless liquid. bp. 91—93 °C (5 mmHg); EI-MS *m/z* (%): 179 (M^+, 7.5), 124 (81.7), 106 (100.0), 77 (44.6), 56 (21.9); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.98 (3H, t, J = 7.19 Hz), 1.49 (2H, m), 1.76 (2H, m), 4.33 (2H, dt, $J_{C\text{-}H}$ = 2.8 Hz, J = 6.7 Hz), 7.44 (2H, m), 7.55 (1H, m), 8.05 (2H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.7; IR (neat) cm^{-1}: 2961, 1678, 1248, 1105.

[α-^{13}C]Benzyl Alcohol

To a stirred suspension of $LiAlH_4$ (0.61 g, 16.1 mmol) in dry THF (9 ml) was added dropwise a solution of butyl [*carbonyl*-^{13}C]benzoate (**10**) (1.92 g, 10.7 mmol) in dry THF (8 ml) at 0 °C over 15 min. The mixture was stirred for 3 h at room temperature, then the reaction was quenched with 40% KOH at 0 °C. The precipitate was filtered off and washed with ether. The combined filtrates were evaporated and the residue was purified by column chromatography on silica gel (hexane : ethyl acetate = 4 : 1) to give [α-^{13}C]benzyl alcohol (1.14 g, 97%) as a colorless liquid.

[α-^{13}C]Benzyl alcohol (5.15 g) was also prepared from [*carbonyl*-^{13}C]benzoic acid (99 atom % ^{13}C, 5.79 g, 47.1 mmol) in quantitative yield by means of the same procedure. EI-MS *m/z* (%): 109 (M^+, 100.0), 92 (14.4), 79 (74.0), 77 (39.1), 51 (14.3); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.75 (1H, brs), 4.69 (2H, dd, J = 5.1 Hz, $J_{C\text{-}H}$ = 142.5 Hz), 7.29—7.37 (5H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 65.3; IR (neat) cm^{-1}: 3333, 3031, 2923, 2858, 1454, 1205, 996.

[α-^{13}C]Benzyl Bromide (4)

A solution of phosphorus tribromide (3.4 ml, 5.10 g, 18.8 mmol) in dry ether (14 ml) was added dropwise to [α-^{13}C]benzyl alcohol (5.15 g, 47.1 mmol) at 0 °C over 30 min with stirring. The mixture was stirred at room temperature for 20 h, and the reaction was quenched with water. The aqueous layer was extracted four times with ether. The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated. The residual crude product was purified by distillation under reduced pressure to give [α-^{13}C]benzyl bromide (**4**) (5.28 g, 65%) as a colorless liquid. bp. 79—81 °C (10 mmHg); EI-MS *m/z* (%): 173 (M^++2, 11.2), 171 (M^+, 11.8), 92 (100.0); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.50 (2H, d, $J_{C\text{-}H}$ = 152.7 Hz), 7.29—7.41 (5H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 33.5; IR (neat) cm^{-1}: 3064, 3031, 2961, 1218, 1200, 1069, 1028.

(3R,5S)-2,3,5,6-Tetrahydro-5-phenyl-3-phenyl[^{13}C]methyl-N-(tert-butyloxy-carbonyl)-4H-1,4-oxazin-2-one (11)

To a stirred solution of the oxazinone **1** (9.92 g, 35.8 mmol) in a mixture of dry THF (14.5 ml) and dry DME (52 ml) was added dropwise sodium bis(trimethylsilyl)amide (1.0 M solution in THF, 37.5 ml, 37.5 mmol) at -78 °C over 30 min. The mixture was stirred for 30 min, then [α-^{13}C]benzyl bromide (**4**) (5.27 g, 30.7 mmol) was added dropwise to it at same temperature over 10 min, and the whole was stirred for 4.5 h. The reaction was quenched with saturated NH_4Cl, and the mixture was partitioned between ether and 3 N HCl. The aqueous layer was extracted five times with ether. The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated. The resulting solid was purified by column chromatography on silica gel (hexane : ethyl acetate = 4 : 1) and crystallized from hexane-benzene to give the phenyl[^{13}C]methyloxazinone **11** (10.59 g, 94 %) as white needles. mp. 178.8—180.6 °C; EI-MS *m/z* (%): 368 (M^+, 19.7), 312 (29.1), 176 (100.0), 130 (19.5), 104 (21.3), 92 (17.5), 57 (83.8); ^{1}H-NMR (400 MHz, $CDCl_3$, 60 °C) δ: 1.17—1.44 (9H, brs), 3.27 (1H, ddd, *J* = 3.6, 13.6 Hz, $J_{C\text{-}H}$ = 130.9 Hz), 3.34—3.73 (2H, m), 4.82 (1H, m), 5.12 (1H, m), 7.01—7.40 (10H, m); ^{13}C-NMR (100 MHz, $CDCl_3$, 60 °C) δ: 39.4; IR (KBr) cm^{-1}: 3032, 2980, 2917, 1746, 1695, 1497, 1454, 1376, 1263, 1172, 1125, 1075, 950.

L-[3-^{13}C]Phenylalanine Hydrochloride (2)

A solution of the phenyl[^{13}C]methyloxazinone **11** (9.00 g, 24.4 mmol) in a saturated solution of HCl in dry ethanol, prepared from HCl gas and dry ethanol, was refluxed for 5.5 h. The resulting solution was evaporated to give crude (2*R*)-*N*-{(1*S*)-2-hydroxy-1-phenylethyl}[3-^{13}C]phenyl-alanine ethyl ester hydrochloride (**12**) (8.65 g, in quantitative yield) as a slightly yellow solid.

A 4.01 g portion of the crude *N*-alkylphenylalanine ester **12** (corresponding to 11.33 mmol) was dissolved in dry ethanol (56 ml), and to the solution was added 10 % Pd-C (3.62 g, 3.40 mmol). The mixture was shaken under a hydrogen atmosphere (6.0 kgf/cm^2) at room temperature. After 20 h, the mixture was filtered through Celite, and the Celite pad was washed with dry ethanol. The combined filtrates were evaporated to give crude L-[3-^{13}C]phenylalanine ethyl ester hydrochloride (**13**) (4.21 g) as a slightly yellow solid.

A suspension of the crude ethyl ester hydrochloride **13** (4.21 g) in 6 N HCl (45 ml) was heated and refluxed for 5 h, and then evaporated. The residue was dissolved in water and the solution was washed with

$CHCl_3$ and ether. The aqueous layer was evaporated. The resulting crude product was purified by crystallization from water-ethanol to give L-[3-^{13}C]phenylalanine hydrochloride (**2**) (1.89 g) as white needles.

The remaining portion of the crude alkyloxazinone **11** (4.58 g, corresponding to 12.93 mmol) gave L-[3-^{13}C]phenylalanine hydrochloride (**2**) (1.97 g) *via* the same reactions as above. The total yield was 78% from the alkyloxazinone **11** in 3 steps. mp. 222.7—225.6 °C; EI-MS *m/z* (%): 166 (M^+, 7.5), 121 (96.8), 104 (14.9), 92 (68.5), 74 (100.0); ^{1}H-NMR (400 MHz, D_2O) δ: 3.19 (1H, ddd, J = 7.7, 14.6 Hz, $J_{C\text{-}H}$ = 131.4 Hz), 3.33 (1H, ddd, J = 5.6, 14.4 Hz, $J_{C\text{-}H}$ = 132.6 Hz), 4.26 (1H, m), 7.28—7.40 (5H, m); ^{13}C-NMR (100 MHz, D_2O) δ: 38.4; IR (KBr) cm^{-1}: 3400—2200 (br), 2965, 1741, 1605, 1484, 1444, 1421, 1262, 1208, 1099.

1-Iodo-4-(methoxymethyloxy)benzene (8b)

To a solution of 4-iodophenol (20.00 g, 90.9 mmol) in dry THF (180 ml) was added 60% NaH (5.09 g, 0.13 mol) at 0 °C. Chloromethyl methyl ether (7.6 ml, 8.05 g, 0.10 mol) was added dropwise to the solution over 7 min at the same temperature. The mixture was stirred for 1.5 h at that temperature, then the reaction was quenched with saturated NH_4Cl. The reaction mixture was washed three times with water. The combined aqueous layers were extracted four times with $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The residual product was purified by column chromatography on silica gel (hexane : ethyl acetate = 16 : 1) to give 1-iodo-4-(methoxymethyloxy)benzene (**8b**) (23.97 g, in quantitative yield) as a colorless liquid. *Anal.* Calcd for $C_8H_9O_2I$: C, 36.39; H, 3.44; Found: C, 36.57; H, 3.40; EI-MS *m/z* (%): 264 (M^+, 60.6), 234 (20.7), 45 (100.0); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.46 (3H, s), 5.15 (2H, s), 6.82 (2H, m), 7.57 (2H, m); IR (neat) cm^{-1}: 2955, 2901, 1587, 1485, 1236, 1154, 1080, 998, 823.

Methyl 4-(Methoxymethyloxy)[carbonyl-^{13}C]benzoate (14)

To a stirred solution of palladium acetate (92 mg, 0.41 mmol) and triphenylphosphine (0.44 g, 1.68 mmol) in dry methanol (4 ml) were added triethylamine (3.4 ml, 2.48 g, 24.6 mmol) and a solution of 1-iodo-4-(methoxymethyloxy)benzene (**8b**) (2.16 g, 8.18 mmol) in dry methanol (5 ml) at room temperature. The mixture was stirred under a [^{13}C]carbon monoxide (99.5 atom % ^{13}C) atmosphere (3 kgf/cm^2) at 120 °C for 24 h, then filtered through Florisil, and the filtrate was evaporated. The residue was dissolved in ether and washed with saturated $NaHCO_3$. The aqueous layer was extracted three times with ether. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The resulting slightly brown liquid was purified by column chromatography on silica gel (hexane : ethyl acetate = 5 : 1) to give methyl 4-(methoxymethyloxy)[*carbonyl*-^{13}C]benzoate (**14**) (1.01 g, 62%) as a colorless liquid. Methyl 4-hydroxy[*carbonyl*-^{13}C]benzoate (**15**) (90 mg, 7%) was also obtained, and was converted to **14** as follows.

To a solution of methyl 4-hydroxy[*carbonyl*-^{13}C]benzoate (**15**) (88 mg, 0.53 mmol) in dry THF (3 ml) was added 60% NaH (25 mg, 0.63 mmol) at 0 °C. Chloromethyl methyl ether (0.04 ml, 47 mg, 0.58 mmol) was added dropwise to the solution over 0.5 min at same temperature. The mixture was stirred for 20 min at that temperature, then the reaction was quenched with saturated NH_4Cl. The mixture was washed three times with water. The combined aqueous layers were extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The residual product was purified by column chromatography on silica gel (hexane : ethyl acetate = 5 : 1) to give **14** (111 mg, in quantitative yield) as a colorless liquid. **14**; EI-MS *m/z* (%): 197 (M^+, 32.3), 166 (10.0), 137 (14.8), 45

(100.0); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.49 (3H, s), 3.91 (3H, d, $J_{C\text{-}H}$ = 13.6 Hz), 5.23 (2H, s), 7.06 (2H, m), 7.99 (2H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.8; IR (neat) cm^{-1}: 2953, 1679, 1607, 1509, 1433, 1318, 1263, 1201, 1154, 1104, 1082. **15**; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.89 (3H, d, $J_{C\text{-}H}$ = 3.3 Hz), 6.86 (2H, m), 7.97 (2H, m).

4-(Methoxymethyloxy)phenyl[^{13}C]methanol

To a stirred suspension of $LiAlH_4$ (0.26 g, 6.81 mmol) in dry THF (3 ml) was added dropwise a solution of methyl 4-(methoxymethyloxy)[*carbonyl*-^{13}C]benzoate (**14**) (1.12 g, 5.67 mmol) in dry THF (10 ml) at 0 °C over 5 min. After 1.5 h at room temperature, the reaction was quenched by addition with 40% KOH at 0 °C. The precipitate was filtered off and washed with ether. The combined filtrates were evaporated and the residue was purified by column chromatography on silica gel (hexane : ethyl acetate = 2 : 1) to give 4-(methoxymethyloxy)phenyl[^{13}C]methanol (0.91 g, 94%) as a colorless liquid. EI-MS *m/z* (%): 169 (M$^+$, 41.1), 139 (15.3), 107 (10.7), 45 (100.0); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.59 (1H, brs), 3.48 (s, 3H), 4.63 (2H, dd, J = 5.6 Hz, $J_{C\text{-}H}$ = 142.7 Hz), 5.18 (2H, s), 7.02—7.05 (2H, m), 7.28—7.31 (2H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 65.0; IR (neat) cm^{-1}: 3391, 2901, 1613, 1588, 1511, 1443, 1407, 1313, 1234, 1199, 1153, 1079, 1005.

1-Bromo[^{13}C]methyl-4-(methoxymethyloxy)benzene (5)

To a stirred solution of lithium bromide (4.60 g, 53.0 mmol) in dry THF (10 ml) were added triethylamine (1.85 ml, 1.34 g, 13.2 mmol) and a solution of 4-(methoxymethyloxy)-phenyl[^{13}C]methanol (0.90 g, 5.30 mmol) in dry THF (10 ml) at room temperature. Methanesulfonyl chloride (0.82 ml, 1.22 g, 10.6 mmol) was added dropwise to the above mixture at -60 °C over 2 min. The reaction mixture was stirred and allowed to warm to 0 °C over 1.5 h, then the reaction was quenched with water. The aqueous layer was extracted four times with ether. The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated. The residual crude product was purified by column chromatography on silica gel (hexane : ethyl acetate = 5 : 1) to give 1-bromo[^{13}C]methyl-4-(methoxymethyloxy)benzene (**5**) (1.12 g, 91%) as a colorless liquid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.48 (3H, s), 4.50 (2H, d, $J_{C\text{-}H}$ = 153.0 Hz), 5.18 (2H, s), 7.01 (2H, m), 7.33 (2H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 33.6.

(3R,5S)-2,3,5,6-Tetrahydro-3-{4-(methoxymethyloxy)phenyl[^{13}C]methyl}-5-phenyl-N-(tert-butyloxycarbonyl)-4H-1,4-oxazin-2-one (16)

To a stirred solution of the oxazinone **1** (1.47 g, 5.30 mmol) in a mixture of dry THF (3.5 ml) and dry DME (12 ml) was added dropwise sodium bis(trimethylsilyl)amide (1.0 M solution in THF, 5.83 ml, 5.83 mmol) at -78 °C over 5 min. The mixture was stirred for 1 h, then 1-bromo[^{13}C]methyl-4-(methoxymethyloxy)benzene (**5**) (1.11 g, 4.79 mmol) was added dropwise to the above solution at the same temperature over 10 min, and the whole was stirred for 7 h. The reaction was quenched with saturated NH_4Cl. The aqueous layer was extracted five times with ether. The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated. The residual mixture was purified by column chromatography on silica gel (hexane : ethyl acetate = 4 : 1) and the product was recrystallized from hexane-benzene to give the title compound **16** (1.03 g, 50 %) as white needles. mp.

111.3—113.2 °C; EI-MS *m/z* (%): 428 (M^+, 22.1), 321 (83.8), 265 (100.0), 235 (24.7), 221 (25.3), 176 (22.6), 152 (70.0), 122 (23.0); ^{1}H-NMR (400 MHz, $CDCl_3$, 60 °C) δ: 1.15—1.44 (9H, brs), 3.21 (1H, ddd, J = 3.3, 13.9 Hz, $J_{C\text{-}H}$ = 131.2 Hz), 3.29—3.67 (2H, m), 3.48 (3H, s), 3.99 (1H, m), 4.85 (1H, m), 5.07 (1H, m), 5.15 (2H, s), 6.99—7.05 (4H, m), 7.12 (2H, m), 7.23—7.34 (3H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 38.6; IR (KBr) cm^{-1}: 3494, 2974, 2931, 1754, 1692, 1612, 1513, 1453, 1357, 1306, 1237, 1117, 1017.

L-[3-^{13}C]Tyrosine (3)

A suspension of the alkylated oxazinone **16** (1.09 g, 2.54 mmol) in a saturated solution of HCl in dry ethanol, prepared from HCl gas and dry ethanol, was refluxed for 2.5 h. The resulting solution was evaporated to give crude (2*R*)-*N*-{(1*S*)-2-hydroxy-1-phenylethyl}[3-^{13}C]tyrosine ethyl ester hydrochloride (**17**) (0.98 g, in quantitative yield) as a slightly yellow solid.

A mixture of the crude *N*-alkyl[3-^{13}C]tyrosine derivative **17** (0.98 g) and 10% Pd-C (1.08 g, 1.01 mmol) in dry ethanol (15 ml) was shaken under a hydrogen atmosphere (6.0 kgf/cm^2) at room temperature. After 50 h, the mixture was filtered through Celite, and the Celite pad was washed with ethanol. The combined filtrates were evaporated to give crude L-[3-^{13}C]tyrosine ethyl ester hydrochloride (1.09 g) as a slightly yellow solid.

A suspension of the crude [3-^{13}C]tyrosine ethyl ester (1.09 g) in 6 N HCl (20 ml) was heated and refluxed for 4 h, and then evaporated. The residue was dissolved in water and the solution was washed with $CHCl_3$ and ether. The aqueous layer was evaporated to give the crude product. Diluted ammonium hydroxide was added to a solution of the prior crude product dissolved in water to adjust the pH to 5.66, affording a precipitate. The precipitate was separated by centrifugation and washed three times with water and once with dry ethanol to give L-[3-^{13}C]tyrosine (**3**) (0.26 g, 58%) as a slightly gray powder. mp. 253.5—256.9 °C; EI-MS *m/z* (%): 182 (M^+, 7.9), 108 (100.0); ^{1}H-NMR (400 MHz, 2 N KOD) δ: 2.33 (1H, ddd, J = 7.2, 13.6 Hz, $J_{C\text{-}H}$ = 128.3 Hz), 2.48 (1H, ddd, J = 5.4, 13.6 Hz, $J_{C\text{-}H}$ = 128.3 Hz), 3.05 (1H, m), 6.23 (2H, m), 6.65 (2H, m); ^{13}C-NMR (100 MHz, 2 N KOD) δ: 41.9; IR (KBr) cm^{-1}: 3400—2400 (br), 3209, 2964, 1608, 1590. 1514, 1413, 1360, 1327, 1263, 1099, 1036.

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