Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 701-708 (1999)

An Asymmetric Synthesis of L-[2-^{13}C]Aspartic Acid from Sodium [2-^{13}C]Acetate

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Summary

L-[2-^{13}C]Aspartic acid was synthesized by using Dellaria's oxazinone labelled with ^{13}C at the 3-position, prepared from phenyl [2-^{13}C]bromoacetate and (*S*)-2-phenylglycinol, as a chiral glycine equivalent. Phenyl [2-^{13}C]bromoacetate was derived from sodium [2-^{13}C]acetate. Alkylation of the [3-^{13}C]oxazinone with ethyl bromoacetate was achieved with high diastereoselectivity. Finally, sequential deprotection and removal of the chiral auxiliary of the alkylated [3-^{13}C]oxazinone afforded L-[2-^{13}C]aspartic acid.

Key words: L-[2-^{13}C]aspartic acid, chiral glycine equivalent, sodium [2-^{13}C]acetate.

Introduction

Stable isotope-labelled amino acids are useful for biological studies.[1] We described previously an asymmetric synthesis of L-[3-^{13}C]phenylalanine and L-[3-^{13}C]tyrosine from [^{13}C]carbon monoxide and Dellaria's oxazinone **1a**[2] as a chiral glycine equivalent.[3] This method is also applicable to other

CCC 0362–4803/99/070701–08$17.50

Received 25 January 1999
Accepted 1 February 1999

amino acids with labelling at other positions. In this paper, we describe an asymmetric synthesis of L-[2-^{13}C]aspartic acid (**2**).

Results and Discussion

Dellaria's oxazinone **1b** labelled with ^{13}C at the 3-position, corresponding to the 2-position of aspartic acid, was synthesized from sodium [2-^{13}C]acetate (**3**) (Fig. 1). Sodium [2-^{13}C]acetate (**3**) was converted to [2-^{13}C]bromoacetyl bromide in two steps.[4] Esterification with phenol of the resulting [2-^{13}C]bromoacetyl bromide gave phenyl [2-^{13}C]acetate (**4**). Formation of the oxazinone ring with **4** and (*S*)-2-phenylglycinol and protection of the amino group with a *t*-butyloxycarbonyl group (Boc) were conducted in one pot to give the [3-^{13}C]oxazinone **1b**, modifying the method of Dellaria, because the oxazinone before protection readily decomposed at over 40 °C and the reproducibility of the reaction was poor.

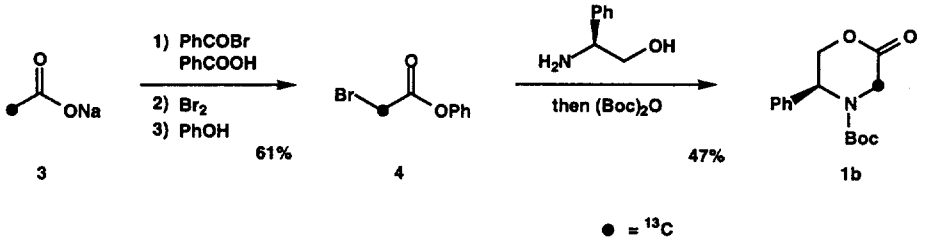
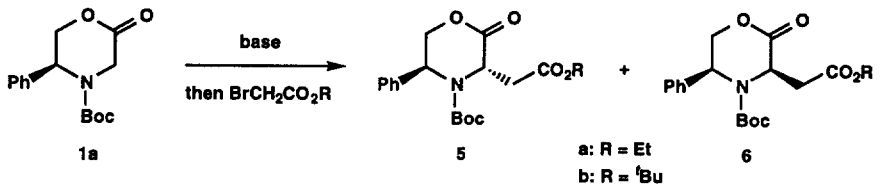


Fig. 1

The conditions for the alkylation of **1b** with ethyl bromoacetate were optimized using the unlabelled oxazinone **1a** (Fig. 2). The results are shown in the table. The reaction using sodium bis(trimethylsilyl)amide (NaHMDS) in THF-DME, the same base and solvents as used for the synthesis of L-[3-^{13}C]phenylalanine and L-[3-^{13}C]tyrosine,[3] proceeded with poor diastereoselectivity and yield. However, the use of lithium bis(trimethylsilyl)amide (LHMDS) as a base in THF (entry 4), as reported by Dellaria and Santarsiero,[5] afforded the desired **5a** with high diastereoselectivity in high yield.



entry	solvent	base	R	yield	ratio (5 : 6)*
1	THF, DME	NaHMDS (1.0 eq)	Et	74%	3.5 : 1
2	THF, DME	NaHMDS (1.0 eq)	^{t}Bu	32% (recovered SM: 29%)	10 : 1
3	THF, DME	NaHMDS (0.98 eq)	Et	51% (recovered SM: 30%)	7 : 1
4	THF	LHMDS (1.0 eq)	Et	quant.	>100 : 1

*The ratio was determined from the ^{1}H-NMR spectra.

Fig. 2

The synthesis of [2-^{13}C]aspartic acid (**2**) was carried out under the above conditions (Fig. 3). Alkylation of **1b** with ethyl bromoacetate gave the desired **5c** with high diastereoselectivity in 85% yield. Finally, ethanolysis of **5c** and hydrogenolysis followed by acid hydrolysis of the resulting diester **7** gave L-[2-^{13}C]aspartic acid (**2**) in 88% yield. The chemical structure of **2** was confirmed by FAB-MS, ^{1}H-NMR, ^{13}C-NMR and IR analyses. The ^{13}C-NMR spectrum of **2** showed the enriched signal at 55.9 ppm, assigned to the 2-position of aspartic acid. The ^{1}H-NMR spectrum showed that **2** was enriched to the extent of over 99 atom% ^{13}C. The optical

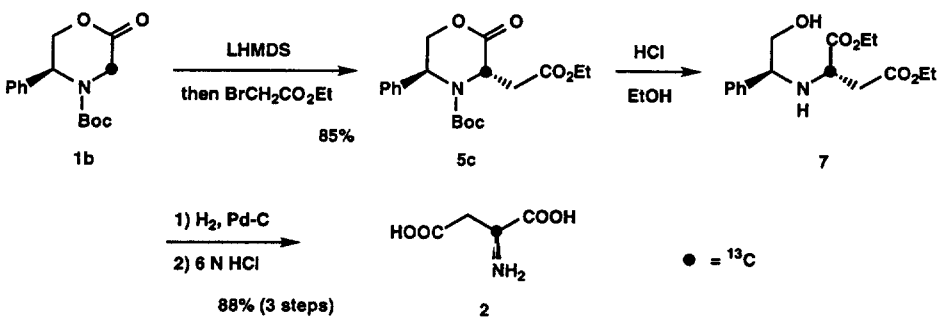


Fig. 3

purity was over 99% ee and the absolute configuration was L, as determined by HPLC-CD analysis using a chiral column.[6]

Thus, the synthesis of L-[2-^{13}C]aspartic acid (**2**) from sodium [2-^{13}C]acetate (**3**) was achieved by alkylation of [3-^{13}C]oxazinone (**5c**), as a chiral glycine equivalent, with ethyl bromoacetate. L-[1-^{13}C]Aspartic acid should be similarly obtainable synthesized from sodium [1-^{13}C]acetate *via* [2-^{13}C]oxazinone. The preparation of ethyl [1 or 2-^{13}C]bromoacetate from sodium [1 or 2-^{13}C]acetate has been reported.[4] Therefore, in this method the use of sodium [1 or 2-^{13}C]acetate as a starting material enables regioselective labelling of optically active aspartic acid.

Experimental

Materials

Sodium [2-^{13}C]acetate (99 atom% ^{13}C) was purchased from Cambridge Isotope Laboratories.

Instruments

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-GSX 400 Fourier-transform spectrometer. The chemical shifts were reported in δ values relative to tetramethylsilane (TMS) at 0 ppm in $CDCl_3$ or HOD at 4.7 ppm in D_2O on ^{1}H-NMR and relative to $CDCl_3$ at 77.0 ppm or 3-(trimethylsilyl)propionic-*2,2,3,3,-d*$_4$ acid sodium salt (TSP) at 0 ppm in D_2O on ^{13}C-NMR. IR spectra were recorded on a JASCO VALOR-III Fourier-transform spectrometer. EI- and FAB-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.

Phenyl [2-^{13}C]bromoacetate (4)

Sodium [2-^{13}C]acetate (**3**, 99 atom% ^{13}C, 3.58 g, 43.3 mmol) and benzoic acid (4.76 g, 39.0 mmol) were stirred *in vacuo* at 50 °C for 3.5 h. To that mixture was added benzoyl bromide (25.5 ml, 216.6 mmol). The whole was heated to 120 °C and stirred overnight, then distilled under reduced pressure into a receiving flask to give [2-^{13}C]acetyl bromide.

Bromine (11.2 ml, 216.6 mmol) was added to the above receiving flask at -78 °C. The mixture was stirred at room temperature for 5 min, and then refluxed for 5 h. Excess bromine was flushed out with Ar gas at 0 °C to give crude [2-^{13}C]bromoacetyl bromide.

To the crude [2-^{13}C]bromoacetyl bromide was added dropwise over 1 h a solution of phenol (4.6 ml, 52.0 mmol) in dry CH_2Cl_2 (35 ml) at room temperature. The mixture was stirred for 1.5 d at that temperature, and was then neutralized with aqueous 30% K_2CO_3. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The residue was distilled under reduced pressure to give phenyl [2-^{13}C]bromoacetate (**4**, 7.23 g, 77%) as a colorless liquid. bp. 110—125 °C (5 mmHg); EI-MS *m/z* (%): 217 (M^++2, 8.4), 215 (M^+, 8.6), 124 (2.3), 122 (2.6), 94 (100), 65 (4.8); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.06 (2H, d, $J_{C\text{-}H}$ = 154.0 Hz), 7.13 (1H, m), 7.27 (2H, m), 7.41 (2H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 25.5; IR (neat) cm^{-1}: 3064, 1755, 1592, 1490, 1473, 1396, 1267, 1190, 1162, 1128, 1070, 1044.

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

To a suspension of (*S*)-2-phenylglycinol (2.83 g, 20.6 mmol) in dry CH_3CN (40 ml) was added *N*,*N*-diisopropylethylamine (8.2 ml, 46.9 mmol) at room temperature. A solution of phenyl [2-^{13}C]bromoacetate (**4**, 4.05 g, 18.7 mmol) in dry CH_3CN (20 ml) was added dropwise to the mixture over 10 min at the same temperature. The reaction mixture was stirred for 5 h, and a solution of $(Boc)_2O$ (6.14 g, 28.1 mmol) in dry CH_3CN (20 ml) was

added. The whole was stirred for 5 h, and then evaporated. The residue was partitioned between $CHCl_3$ and saturated $NaHCO_3$. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The resulting product was purified by column chromatography on silica gel (hexane : ethyl acetate = 3 : 1) and crystallized from ethyl acetate-hexane to give the title product **1b** (2.47 g, 47%) as white needles. mp. 83.7—85.0 °C; EI-MS *m/z* (%): 278 (M^+, 1.7), 234 (9.6), 222 (14.6), 205 (9.8), 178 (76.6), 119 (83.5), 104 (31.5), 92 (27.2), 57 (100.0); ^{1}H-NMR (400 MHz, $CDCl_3$, 55 °C) δ: 1.25—1.46 (9H, brs), 4.19 (1H, dd, J = 18.1 Hz, $J_{C\text{-}H}$ = 176.6 Hz), 4.47 (1H, dd, J = 5.9, 12.1 Hz), 4.55 (1H, dd, J = 18.1 Hz, $J_{C\text{-}H}$ = 177.4 Hz), 4.57 (1H, dd, J = 4.1, 12.1 Hz), 5.12 (1H, m), 7.23—7.40 (5H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 44.4; IR (KBr) cm^{-1}: 2979, 2930, 1759, 1747, 1694, 1452, 1400, 1376, 1293, 1233, 1197, 1167, 1118, 1070.

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

To a stirred solution of the [3-^{13}C]oxazinone **1b** (1.08 g, 3.87 mmol) in dry THF (25 ml) was added dropwise over 7 min lithium bis(trimethylsilyl)amide, prepared from 1,1,1,3,3,3-hexamethyldisilazane (0.90 ml, 4.25 mmol) and *n*-butyllithium (3.02 M solution in hexane, 1.34 ml, 4.06 mmol) in dry THF (5 ml), at -78 °C. After 1 h, ethyl bromoacetate (0.45 ml, 4.06 mmol) was added dropwise over 1 min to the above mixture at the same temperature, and the whole was stirred for 1.5 h. The reaction was quenched with saturated NH_4Cl. 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 resulting solid was purified by column chromatography on silica gel (hexane : ethyl acetate = 2 : 1) and crystallized from hexane-benzene to give **5c** (1.22 g, 87%) as white needles. mp. 99.6—103.7 °C; EI-MS *m/z* (%): 364 (M^+, 5.8), 308 (8.2), 291 (7.0), 264 (67.5), 219 (10.9), 191 (10.6), 176 (7.9), 133 (13.6), 118 (7.1), 104 (100.0), 91 (5.4); ^{1}H-NMR (400 MHz, $CDCl_3$, 60 °C)

δ: 1.23—1.45 (9H, brs), 1.28 (3H, t, J = 7.2 Hz), 3.08 (1H, dt, J = 15.7 Hz, $J_{CC\text{-}H}$ = J = 4.9 Hz), 3.20 (1H, ddd, $J_{CC\text{-}H}$ = 4.9 Hz, J = 6.4, 15.7 Hz), 4.19 (2H, q, J = 7.2 Hz), 4.42 (1H, dd, J = 1.8, 11.8 Hz), 4.99 (1H, dd, $J_{CC\text{-}H}$ = 3.1, 11.8 Hz), 5.05 (1H, ddd, J = 4.9, 6.4 Hz, $J_{C\text{-}H}$ = 147.6 Hz), 5.07 (1H, br), 7.09 (2H, m), 7.26—7.34 (3H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 53.5; IR (KBr) cm^{-1}: 2979, 2939, 1752, 1694, 1478, 1377, 1299, 1248, 1208, 1186, 1161, 1128, 1074, 1044, 969.

L-[2-^{13}C]Aspartic Acid (2)

A solution of the alkylated [3-^{13}C]oxazinone **5c** (1.02 g, 2.79 mmol) in a saturated solution of HCl in dry ethanol (16 ml), prepared by bubbling HCl gas into dry ethanol, was refluxed for 5 h. The resulting solution was evaporated to give crude diethyl (2*S*)-2-{[(1*S*)-2-hydroxy-1-phenylethyl]amino}[2-^{13}C]butanedioate hydrochloride (**7**, 1.18 g) as a slightly yellow solid.

The crude *N*-alkylaspartic acid diester hydrochloride **7** was dissolved in dry ethanol (15 ml), and to this solution was added 10% Pd-C (0.89 g, 0.84 mmol). The mixture was shaken under a hydrogen atmosphere (6.0 kgf/cm^2) at room temperature. After 1.5 d, the mixture was filtered through Celite, and the Celite pad was washed with dry ethanol. The combined filtrates were evaporated to give crude L-[2-^{13}C]aspartic acid diethyl ester hydrochloride (0.94 g) as a brown solid

A suspension of the crude ethyl ester hydrochloride in 6 N HCl (14 ml) was heated to 85 °C and stirred for 5 h. The mixture was washed with $CHCl_3$ and ether. The aqueous layer was evaporated. The resulting crude aspartic acid hydrochloride was purified by precipitation from dry ethanol with dry pyridine[7] to give L-[2-^{13}C]aspartic acid (**2**, 0.33 g, 88% in 3 steps) as a white powder. mp. 309.8 °C (decomp.); FAB-MS (glycerol) *m/z*: 135 (MH^+); ^{1}H-NMR (400 MHz, 2 N KOD) δ: 2.05 (1H, ddd, $J_{CC\text{-}H}$ = 4.6 Hz, J = 9.5, 15.4 Hz), 2.39 (1H, dt, J = 15.4 Hz, $J_{CC\text{-}H}$ = J = 3.9 Hz), 3.29 (1H, ddd, J = 3.9, 9.5 Hz,

$J_{C\text{-}H}$ = 138.6 Hz); ^{13}C-NMR (100 MHz, 2 N KOD) δ: 55.9; IR (KBr) cm^{-1}: 3200—2400 (br), 2964, 2729, 1692, 1648, 1601, 1509, 1420, 1327, 1301, 1261, 1154, 1099, 1040, 802.

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