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Research Article

An asymmetric synthesis of L-[3-^{13}C]alanine

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Summary

L-[3-^{13}C]Alanine was synthesized from [^{13}C]methyl iodide by using Dellaria's oxazinone, prepared from phenyl[2-^{13}C]bromoacetate and (*S*)-2-phenylglycinol, as a chiral glycine equivalent. Alkylation of the oxazinone with [^{13}C]methyl iodide was achieved with high diastereoselectivity. Hydrolysis and removal of the chiral auxiliary of the alkylated oxazinone gave L-[3-^{13}C]alanine. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: L-[3-^{13}C]alanine; [^{13}C]methyl iodide; chiral glycine equivalent; oxazinone

Introduction

Stable isotope-labelled amino acids are useful for the diagnosis of disease, the study of the biosynthesis of natural products and other biological studies.[1–3] We described previously asymmetric syntheses of L-[3-^{13}C]phenylalanine, L-[3-^{13}C]tyrosine[4] and L-[2-^{13}C]aspartic acid[5] from Dellaria's oxazinone **1**[6] or its ^{13}C-labelled form as a chiral glycine equivalent. This method is also applicable to the synthesis of various amino acids with labelling at other positions. In this paper, we describe an asymmetric synthesis of L-[3-^{13}C]alanine (**2**) (For a recent synthesis of optically active ^{13}C-labelled alanine from a chiral glycine equivalent, see ref. 7 and 8.).

Results and discussion

Dellaria's oxazinone **1** was prepared from (*S*)-2-phenylglycinol and bromoacetyl bromide by modifying the method of Dellaria, described in a previous report.[5] Diastereoselective alkylation of **1** was performed by treatment of **1** with sodium bis(trimethylsilyl)amide (NaHMDS) in THF-DME and addition of [^{13}C]methyl iodide to the resulting enolate of **1** to give the alkylated

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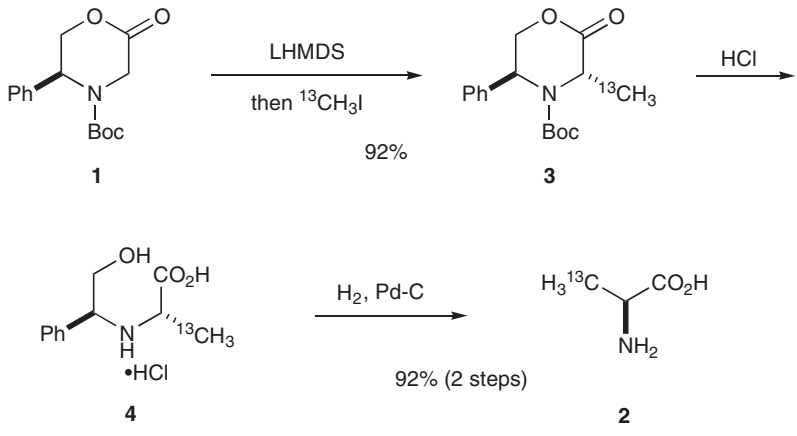

Scheme 1.

oxazinone **3** in 92% yield with high diastereoselectivity (Scheme 1). Hydrolysis of the oxazinone ring and removal of the Boc group of **3** were simultaneously carried out by heating at 80°C in 6 M HCl to afford *N*-alkylated L-[3-^{13}C]alanine **4**, which was used in the next step without purification. Finally, hydrogenolysis of **4** gave L-[3-^{13}C]alanine (**2**) in 92% yield from **3**. The overall yield from [^{13}C]methyl iodide to L-[3-^{13}C]alanine (**2**) was 84%.

The ^{13}C-NMR spectrum of **2** showed the enriched signal at 19.0 ppm, assigned to the 3-position of alanine. The ^{1}H-NMR spectrum showed a strong signal at 1.49 ppm as a double-doublet (J_{C-H} = 129.9 Hz and J_{H-H} = 7.2 Hz), assigned to the 3-position of **2**, and a weak signal as a doublet (J_{H-H} = 7.2 Hz), assigned to the same position of residual unlabelled alanine. The enrichment ratio was calculated at 99 atom% ^{13}C or above, based on the integration ratio of these signals. The optical purity was over 98% ee and the absolute configuration was L, as determined by HPLC analysis using a chiral column.[9]

Thus, the synthesis of L-[3-^{13}C]alanine (**2**) from [^{13}C]methyl iodide was accomplished by diastereoselective alkylation of Dellaria's oxazinone **1**. The D-form of **2** and other ^{13}C-isotopomers of L- or D-alanine should be similarly obtainable from the enantiomer of **1**, which could be derived from (*R*)-phenylglycinol, and ^{13}C-oxazinone, which we have previously prepared from sodium [1- or 2-^{13}C]acetate.[5]

Experimental

[^{13}C]Methyl iodide (99 atom% ^{13}C) was supplied by Cambridge Isotope Laboratories. Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GSX-400 Fourier-transform spectrometer. The chemical shifts were

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reported in δ values relative to tetramethylsilane (TMS) at 0 ppm in $CDCl_3$ or sodium 3-trimethylpropionate-d_4 (TSP) at 0 ppm in D_2O on ^{1}H-NMR and relative to $CDCl_3$ at 77.0 ppm or 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 HR-FAB-MS were obtained with a JEOL JMS-DX-302 double-focusing spectrometer and a Finnigan Autospec double-focusing spectrometer, respectively. HPLC analysis was carried out on a JASCO 800 Series HPLC system with a JASCO 875-UV detector. The column was Crown Pak CR(-) (150 mm × 4 mm i.d.), purchased from Daicel. HPLC analysis was performed at 20°C, and the other conditions were as described in a previous report.[9]

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

To a stirred solution of the oxazinone **1** (4.5 g, 16.2 mmol) in a 1:1 mixture of THF and DME (54 ml) was added dropwise a 1 M solution of NaHMDS in THF (27.6 ml, 27.6 mmol) over 30 min at −78°C, and the mixture was stirred for 2 h. [^{13}C]Methyl iodide was added dropwise to the resulting enolate solution. The mixture was stirred for 1 h at that temperature, then poured into saturated NH_4Cl with vigorous stirring. The aqueous layer was extracted with ether 3 times. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. The residue was crystallized from hexane AcOEt to give **3** (4.74 g) in 92% yield. mp. 145.6–147.4°C; ^{1}H-NMR ($CDCl_3$, 50°C) δ: 1.31 (brs, 9H), 1.63 (dd, J_{C-H} = 130.4 Hz, J = 7.2 Hz, 3H), 4.45 (d, J = 11.8 Hz, 1H), 4.75 (dd, J = 3.1, 11.8 Hz, 1H), 4.91 (m, 1H), 5.09 (m, 1H), 7.10 (m, 2H), 7.25–7.39 (m, 3H). ^{13}C-NMR ($CDCl_3$) δ: 20.0. IR (KBr) cm^{-1}: 3030, 3000, 3965, 1754, 1680, 1495, 1398, 1370, 1355, 1297, 1160, 1120, 1068, 1039, 748, 739, 700. EI-MS (%) m/z: 292 (M^+, 9.3), 236 (18.1), 192 (16.9), 147 (14.8), 133 (12.7), 104 (68.4), 57 (100).

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

A solution of the ^{13}C-methyloxazinone **3** in 6 M HCl was heated at 80°C for 2 h. After lyophilization, the residue was dissolved in EtOH (40 ml), and 10% Pd–C (4.3 g, 4.1 mmol) was added. The mixture was shaken for 2 d under hydrogen (5 kg/cm^2). The catalyst was removed by filtration through Celite, and the filtrate was evaporated. The residue was applied to a DOWEX 50W X8 ion-exchange column with EtOH. The column was washed with deionized water. The desired product was eluted with 1 M NH_4OH. The eluate was lyophilized, and the residue was crystallized from water–EtOH to give L-[3-^{13}C]alanine (**2**, 0.83 g) in 92% yield. mp. 226.2–227.7°C. Rt = 5.2 min (D-form gave Rt = 6.3 min, which was not detected). ^{1}H-NMR (D_2O) δ: 1.49

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(dd, (J_{C-H} = 129.9 Hz, J = 7.2 Hz, 3H), 3.79 (dq, J_{C-H} = 4.6 Hz, J = 7.2 Hz, 1H). ^{13}C-NMR (D_2O) δ: 19.0. IR (KBr) cm^{-1}: 3082, 2602, 1623, 1591, 1455, 1411, 1359, 1306, 1233, 1153, 1110, 1012, 915, 847, 771, 648. HR-FAB-MS (glycerol): Calculated for $C_2^{13}C_1H_8NO_2$: 91.0586. found: 91.0592 (MH^+).

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