SYNTHESIS OF [6-^{13}C]-L-LYSINE

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SUMMARY

A short and efficient enantioselective synthesis of [6-^{13}C]-L-lysine from commercially available N-benzyloxycarbonyl-L-glutamic acid α-methyl ester is described using [^{13}C]-sodium cyanide as the source of isotopic label.

Key words : L-lysine, carbon-13, enantioselective synthesis

INTRODUCTION

In order to faciliate a comparison of collagen matrices in normal and diseased ocular tissue by NMR spectroscopy, [6-^{13}C]-L-lysine is required. Lysyl oxidase catalyses the oxidative deamination of lysine to give δ-glutamate semialdehyde which then undergoes a condensation reaction to give cross-links which help to stabilise collagen and give it remarkable tensile strength[1]. Since collagen represents the major component of ocular matrices, it is a prime target for research into disorders such as glaucoma and myopia[2].

A valuable method for the preparation of [6-^{13}C]-L-lysine has been described[3] using the bis-lactim ether of cyclo(D-Val-Gly) and [^{13}C]-sodium cyanide as the source of isotopic label . A disadvantage of this strategy is the introduction of the label at the first stage of the synthesis. We now describe an alternative approach for the preparation of [6-^{13}C]-L-lysine from commercially available N-benzyloxycarbonyl-L-glutamic acid α-methyl ester **1** in which [^{13}C]-sodium cyanide is used at a later stage of the synthesis for the efficient utilisation of isotopic label.

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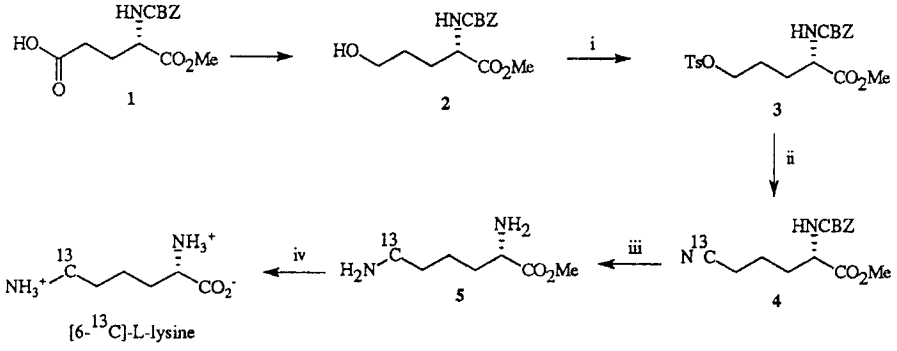
RESULTS AND DISCUSSION

The first stage of the synthesis of [6-^{13}C]-L-lysine shown in Scheme 1 requires the chemoselective reduction of the carboxylic acid of N-benzyloxycarbonyl-L-glutamic acid α-methyl ester **1** to the alcohol **2**. It has been reported that carboxylic acids may be directly converted to alcohols using N,N-dimethylchloromethyleniminium chloride and sodium borohydride[4]. Reaction of **1** under these conditions gave a single product, N-benzyloxycarbonyl-pyroglutamate methyl ester **7** (Scheme 2). Cyclisation of glutamic acid is a facile reaction[5] and formation of the pyroglutamate **7** was presumed to be via an intramolecular cyclisation of the intermediate iminium salt **6**. In order to investigate whether the protecting group would effect the outcome of this reaction, N-^{t}butyloxycarbonyl-L-glutamic acid α-^{t}butyl ester **9** was treated with N,N-dimethylchloromethyleniminium chloride and sodium borohydride and the pyroglutamate **11** was the sole product (81% yield).

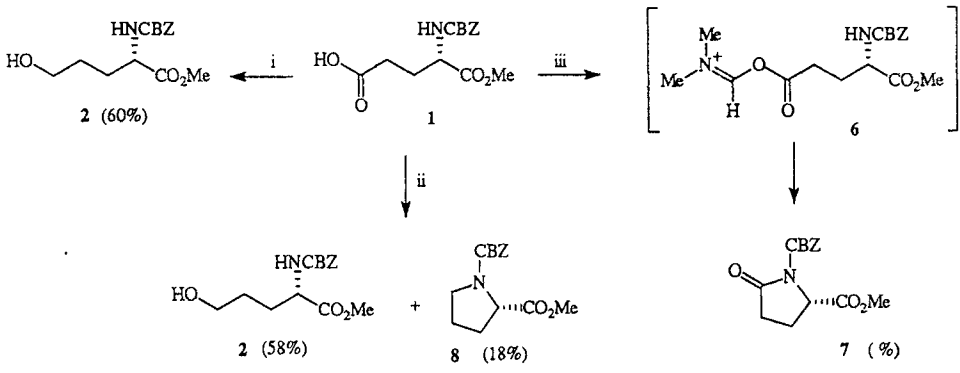


Reagents: i) TsCl, pyridine; ii) Na^{13}CN, DMF; iiii) H_2, PtO; iv) 6N-HCl.

Scheme 1. Preparation of [6-^{13}C]-L-lysine

Alternative methods for the chemoselective reduction of the carboxylic acid **1** to the alcohol **2** were then examined. Activation of the acid as the mixed anhydride by treatment of **1** with ethyl chloroformate followed by reduction with sodium borohydride[7] gave the required alcohol **2** as the sole product in 60% yield (Scheme 2). A similar yield (58%) of alcohol **2** was obtained on reduction of the acid **1** with diborane[8] and the CBZ-L-proline methyl ester **8** (18% yield) was formed as a by-product. Reduction of the N-Boc ^{t}butyl ester **9** with diborane gave the cyclised material **12** as the major product (56% yield) and only a minor amount of the alcohol **10** was isolated. This latter ratio of alcohol to cyclised product was in accord with the

previously reported[9] reduction of the N-Boc hydroxamate derivative which gave 19% yield of the corresponding alcohol and 40% yield of the L-proline derivative.

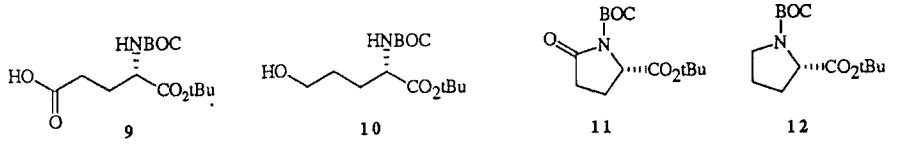


Reagents : i) $ClCO_2Et$, Et_3N, $NaBH_4$; ii) B_2H_6; iii) $(COCl)_2$, DMF, $NaBH_4$

Scheme 2

The next stage of the synthetic sequence involved activation of the alcohol as the tosylate **3** by treatment of **2** with p-toluenesulfonyl chloride in pyridine (Scheme 1). A range of conditions were examined for the nucleophilic displacement of the tosylate with [^{13}C]-cyanide and the highest yield (55%) of the required nitrile **4** was obtained using [^{13}C]sodium cyanide in DMF at room temperature. CBZ-L-proline methyl ester **8** was formed as a by-product (35% yield). A similar ratio of products was obtained on the addition of 18-crown-6 to the reaction mixture or with ultrasound.

To complete the synthesis of [6-^{13}C]-L-lysine, the nitrile **4** was reduced by catalytic hydrogenation over platinum oxide at 50psi to give the amine **5** and the methyl ester was hydrolysed using 6M-hydrochloric acid. Purification of the product by ion exchange chromatography gave the homochiral target compound in quantitative yield from **4**, [6-^{13}C]-L-lysine $[\alpha]_D$ +18.5 (c 2.4, 6N HCl); unlabelled sample from Sigma $[\alpha]_D$ +19.6, (c 1.8, 6N HCl).



In conclusion the 5 stage synthesis of [6-^{13}C]-L-lysine from the commercially available protected glutamic acid utilises the isotopic label to good effect. The source of carbon-13 is the

relatively inexpensive sodium cyanide and once introduced into the nitrile **4**, only two transformations are required which lead to the target [6-^{13}C]-L-lysine in quantitaive yield.

EXPERIMENTAL

^{1}H NMR spectra were recorded as solutions in $CDCl_3$ unless otherwise stated, using tetramethylsilane as the internal reference. The spectra were recorded on a Jeol GX270 MHz or GX400 MHz spectrometers. Probe mass spectra (both high and low resolutions) were recorded on an A.E.I MS9 mass spectrometer and optical rotations were determined as solutions in chloroform, distilled water, or 6N hydrochloric acid (as appropriate) irradiating with the sodium D line (λ = 589nm) using a Perkin Elmer 241 MC polarimeter.

Reduction of N-CBZ-L-Glutamic Acid α-Methyl Ester **1**.

Oxalyl chloride (0.5 ml) was added to a solution of N,N-dimethylformamide (0.85 ml, 0.011mol) in dichloromethane (3 ml) at 0°C and allowed to stir for approximately one hour at room temperature. After removing the solvent, acetonitrile (5 ml) and tetrahydrofuran (7 ml) were added to the residual yellow powder. N-CBZ-L-glutamic acid α- methyl ester **1** (0.291g, 0.001mol) in tetrahydrofuran (5ml) was then added to the reaction mixture at -30°C. After one hour sodium borohydride (0.38g, 0.01mol) was added at -30°C and the temperature of the reaction vessel was slowly raised to room temperature and left for three hours. The reaction mixture was then quenched with 2M hydrochloric acid (20 ml), extracted with diethyl ether (2*20 ml), washed with sodium hydrogen carbonate (20 ml), and then finally dried ($MgSO_4$). The ether was then removed under vacuum. Purification of the product by flash chromatography eluting with 25% ethyl acetate in light petroleum gave N-benzyloxycarbonyl-pyroglutamate methyl ester **7** as a colourless oil (0.18g, 66%). (Found: $[MH]^+$, 278.1029. $C_{14}H_{16}O_5N$ requires $[MH]^+$, 278.1028); $[\alpha]_D$ -26 (c 2.0, $CHCl_3$); δ_H(270MHz): 2.30-2.70(4H, m, CH_2CH_2), 3.60(3H, s, COOMe), 4.70(1H, dd, *J*10, *J*2, 2-H), 5.25(2H, 2*d, each *J*12, PhCH_2), and 7.17(5H, m, Ph); m/z(C.I): 278($[MH]^+$, 37%), 262(11), 234(30), 281(29), 144(39), 119(21), and 91(100).

Reduction of N-^{t}BOC-L-Glutamic Acid α-^{t}Butyl Ester **9**.

The above procedure was repeated on N-^{t}BOC-L-glutamic acid α-^{t}butyl ester **9** (0.291g, 0.001mol) giving N-^{t}butyloxycarbonyl-pyroglutamate ^{t}butyl ester **11** as a colourless oil

(0.22g, 81%). (Found: $[MH]^+$, 286.1658. $C_{14}H_{24}NO_5$ requires $[MH]^+$, 286.1654); $[\alpha]_D$ -38 (c 1.0, $CHCl_3$), Lit.[9] $[\alpha]_D$ -35 (c 1.0, $CHCl_3$); δ_H(270MHz): 1.41(9H, s, COO^tBu), 1.43(9H, s, ^{t}BOC), 1.90-2.50(4H, m, CH_2CH_2), and 4.41(1H, dd, *J*9, *J*3, 2–H); m/z(C.I.): 286($[MH]^+$, 31.34%), 242(23), 230(91), 211(15), and 186(100).

Synthesis of (*S*)-N-CBZ-2-Amino-5-hydroxypentanoic Acid Methyl Ester **2.**

Ethyl chloroformate (0.1g, 0.9mmol) was added at -10°C to a stirred solution of N-CBZ-L-glutamic acid α-methyl ester **1** (0.065g, 0.2mmol) and triethylamine (0.1g, 0.9mmol) in tetrahydrofuran (10ml). The mixture was stirred at -10°C for 0.5 hours. The precipitated mass was filtered and the filtrate was added over a period of 0.25 hours to a solution of sodium borohydride (0.2g, 5mmol) in a mixture of water/THF (2:10ml) at 0°C. The reaction mixture was stirred at room temperature for two hours, acidified with 2M hydrochloric acid (10ml), and extracted with ethyl acetate (2*10ml). The organic layer was washed with aqueous sodium hydrogen carbonate (20ml), dried over sodium sulphate, and then concentrated *in vacuo*. Purification by flash chromatography eluting with 55% ethyl acetate in light petroleum gave the alcohol **2** as a colourless oil (0.037g, 60%). (Found: $[MH]^+$, 282.1341. $C_{14}H_{19}NO_5$ requires $[MH]^+$, 282.1341); $[\alpha]_D$ +8.7 (c 3.0, $CHCl_3$); δ_H(270MHz): 1.50-2.00(4H, m, CH_2CH_2), 3.65(2H, t, *J*6, 5-CH_2), 3.75(3H, s, COOMe), 4.43(1H, dd, *J*18, *J*5, 2–H), 5.15(2H, s, PhCH_2), 5.50(1H, d, *J*8, NH), and 7.35(5H, s, Ph); m/z(C.I.): 282($[MH]^+$, 2%), 262(11), 238(27), 218(43), 206(6), and 91(100).

Synthesis of (*S*)-N-^{t}BOC-2-Amino-5-hydroxypentanoic Acid ^{t}Butyl Ester **10.**

The above procedure was repeated on N-^{t}BOC-L-glutamic acid α-^{t}butyl ester **9** (0.1g, 0.3mmol). Purification of the product by flash chromatography eluting with 55-65% ethyl acetate in light petroleum gave the alcohol **10** as a colourless oil (0.058g, 61%). (Found: $[MH]^+$, 290.1968. $C_{14}H_{28}NO_5$ requires $[MH]^+$, 290.1967); $[\alpha]_D$ +12 (c 2.4, $CHCl_3$), Lit.[9] $[\alpha]_D$ +8.7 (c 2.0, $CHCl_3$); δ_H(270MHz): 1.44(9H, s, COO^tBu), 1.47(9H, s, ^{t}BOC), 1.54-1.90(4H, m, 3-CH_2CH_2), 3.68(2H, t, *J*6, 5-CH_2), 4.21(1H, d, *J*7, 2-H), and 5.17(1H, d, *J*7, NH); m/z(C.I.): 290($[MH]^+$, 51%), 234(39), 225(28), 190(28), and 178(100).

Synthesis of (*S*)-N-CBZ-2-Amino-5-hydroxypentanoic Acid Methyl Ester **2** using Diborane.

Sodium borohydride (0.114g, 0.003mol) in diglyme (3 ml, dried from calcium hydride) was allowed to drop into a flask containing stirring boron trifluoride etherate (2 ml) under nitrogen to form diborane gas. The diborane gas was then flushed into a second flask containing a stirring solution of N-CBZ-L-glutamic acid α-methyl ester **1** (0.2g, 0.7mmol) in tetrahydrofuran (10 ml). After thirty minutes the resulting boron complex was hydrolysed with distilled water (20 ml) and the resulting solution acidified with 2M hydrochloric acid (5 ml). The solution was then extracted with ethyl acetate, dried ($MgSO_4$), and then concentrated. Purification of the mixture by flash chromatography eluting with 25-30% ethyl acetate in light petroleum gave N-CBZ-L-proline methyl ester **8** (0.03g, 18%) as a colourless oil. (Found: $[M]^+$, 263.1150. $C_{14}H_{17}NO_4$ requires $[M]^+$, 263.1157); $[\alpha]_D$ -30 (c 2.0, $CHCl_3$). The compound appeared as a 1:1 mixture of rotamers by ^{1}H-NMR. δ_H(270MHz): 1.80-2.25(4H, m, CH_2CH_2), 3.45(2H, m, 5-CH_2), 3.60 and 3.75(3H, 2*s, COOMe), 4.40(1H, m, 2–H), 5.10 and 5.15(2H, 2(2*d), *J*12, PhCH_2), and 7.30-7.36(5H, m, Ph); m/z(C.I.): 263($[M]^+$, 6%), 236(1), 231(1), 204(26), 160(27), and 91(100). Further elution with 55% ethyl acetate in light petroleum returned the desired alcohol **2** (0.11g, 58%) as a colourless oil. Spectral data as before.

Synthesis of (*S*)-N-^{t}BOC-2-Amino-5-hydroxypentanoic Acid α-^{t}Butyl Ester **10** using Diborane.

The above procedure was repeated on N-^{t}BOC-L-glutamic acid α-^{t}butyl ester **9** (0.2g, 0.7mmol). The reaction mixture was purified by flash chromatography. Eluting with 28% ethyl acetate in light petroleum gave N-^{t}BOC-L-proline ^{t}butyl ester **12** as a colourless oil (0.1g, 58%). (Found: $[MH]^+$, 272.1869. $C_{14}H_{26}NO_4$ requires $[MH]^+$, 272.1862); $[\alpha]_D$ -40 (c 1, $CHCl_3$), Lit.[9] $[\alpha]_D$ -49 (c 1, $CHCl_3$). The compound appeared as a 1:1 mixture of rotamers by ^{1}H NMR. δ_H(270MHz): 1.44(9H, s, COO^{t}Bu), 1.45 and 1.46(9H, 2*s, ^{t}BOC), 1.70-2.30(4H, m, 3-CH_2CH_2), 3.42(2H, m, 5-CH_2), and 4.20(1H, m, 2–H); m/z(C.I.): 272($[MH]^+$, 4%), 243(2), 216(7), 188(10), 170(8), and 160(100). Furthur elution with 55-65% ethyl acetate in light petroleum gave the alcohol **10** as a colourless oil (0.036g, 19%). Spectral data as before.

Synthesis of (*S*)-N-CBZ-2-Amino-5-(*p*-toluenesulfonyloxy)pentanoic Acid Methyl Ester 3.

(*S*)-N-CBZ-2-amino 5-hydroxypentanoic acid α-methyl ester **2** (0.26g,0.96mmol) was added to dry pyridine (5 ml) and allowed to stir for 0.25 hours producing a yellow solution. *p*-Toluenesulfonyl chloride (0.34g, 1.8mmol) was added and the solution was allowed to stir overnight. The resulting black solution was then quenched with distilled water (5 ml), acidified with 2M hydrochloric acid (3 ml), extracted with ethyl acetate (2*10 ml), and dried ($MgSO_4$). The solvent was removed under vacuum and the product purified by flash chromatography. Elution with 20% ethyl acetate in light petroleum gave the tosylate **3** as a colourless oil (0.32g, 84%) .(Found: $[M]^+$, 435.1351. $C_{21}H_{25}O_7NS$ requires $[M]^+$, 435.1351); $[\alpha]_D$ -20 (c 3.8, $CHCl_3$); δ_H(270MHz): 1.80-2.00(4H, m, 2-CH_2CH_2), 2.45(3H, s, $CH_3C_6H_4SO_2$), 3.75(3H, s, COOMe), 4.05(2H, m, 5-CH_2), 4.35(1H, m, 2-H), 5.05(2H, s, $PhCH_2$), 5.15(1H, d, *J*8, NH), 7.16 and 7.80(4H, 2*d, each *J*7, $CH_3C_6H_4SO_2$), and 7.18(5H, s, Ph); m/z(E.I., 70eV): 435(M^+, 3%), 376(4), 332(15), 160(13), 128(8), 108(16), and 91(100).

Synthesis of [6-^{13}C]-(*S*)-N-CBZ-2-Amino-5-cyanopentanoic Acid Methyl Ester 4.

The tosylate **3** (0.7g, 1.6mmol) was dissolved in DMF (5ml) and carefully added to sodium [^{13}C]-cyanide (0.17g, 4.4mmol) in DMF (4ml) at room temperature under a nitrogen atmosphere. After twenty four hours, the reaction was quenched by the addition of aqueous sodium hydrogen carbonate (10ml) with the resulting mixture extracted using diethyl ether (2*20ml). The solvent was dried, removed under vacuum and the product purified by flash chromatography. Elution with 22-24% ethyl acetate in light petroleum gave the nitrile **4** (55%) as a colourless oil. (Found $[MH]^+$, 292.1364. $C_{14}{}^{13}C_1H_{18}N_2O_4$ requires $[MH]^+$, 292.1378); $[\alpha]_D$ +16 (c 4.7, $CHCl_3$); δH(270MHz): 1.60-2.00(4H, m, 3-CH_2CH_2), 2.40(2H, m, 5-CH_2), 3.79(3H, s, COOMe), 4.40(1H, m, 2-H), 5.05(2H, s, $PhCH_2$), 5.20(1H, m, NH), and 7.25(5H, m, Ph); m/z(C.I.): 292($[MH]^+$, 9%), 276(16), 262(1), 248(31), 158(7), 119(10), and 91(100). Furthur elution with 25-30% ethyl acetate in light petroleum returned N-CBZ L-proline α-methyl ester **8** (35%). Spectral data as before.

Synthesis of [6-^{13}C]-L-Lysine

The nitrile **4** (0.25g,0.8mmol) was dissolved in iso-propanol (300ml), concentrated hydrochloric acid (3ml) and a catalytic amount of platinum oxide. The mixture was

hydrogenated at 50psi for two hours. The mixture was filtered through celite and the solvent removed in vacuo to give the amine **5** (0.12g, 100%) as a white solid which was used without further purification. δH(270MHz, H_2O): 1.40-2.00(6H, m, $(CH_2)_3$), 2.98(2H, dt, *J*151, *J*6, 6-$^{13}CH_2$), 3.80(3H, s, COOMe), and 4.10(1H, t, *J*6, 2-H).

[6-^{13}C]-L-lysine methyl ester **5** (0.14g, 0.9mmol) was dissolved in 6M hydrochloric acid (10ml) and heated under reflux overnight under a nitrogen atmosphere. After evaporation of the solvent, the yellow residue was redissolved in water and treated with a small amount of charcoal to decolourise the solution. After filtration of the charcoal, and evaporation, the residue was washed through a Dowex 50W column using concentrated ammonia solution. Evaporation of the solvent gave [6-^{13}C]-L-lysine (0.125g, 100%) as a white solid. $[\alpha]_D$ +18.5 (c 4.4, 6N HCl), unlabelled sample from Sigma $[\alpha]_D$ +19.6 (c 1.8, 6N HCl). δ_H(270MHz, D_2O): 1.50-2.10(6H, m, 3-$CH_2C_2H_4$), 3.10(2H, dt, *J*144, *J*6, 6-$^{13}CH_2$), and 3.90(1H, m, 2–H); m/z(C.I.): 148($[MH]^+$, 1%), 147(3), 134(22), 131(25), 114(10), 113(10), and 86(100).

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