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Research Article

Asymmetric synthesis of L-[4-^{13}C]lysine by alkylation of oxazinone derivative as a chiral glycine equivalent

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Summary

L-[4-^{13}C]Lysine (**2**) was synthesized from sodium [2-^{13}C]acetate (**3**) and Dellaria's oxazinone **1** as a chiral glycine equivalent. Wittig reaction of the glycinal **7** and ^{13}C-labeled phosphonium ylide **5**, prepared from sodium [2-^{13}C]acetate (**3**), gave the α, β-unsaturated ester **8**. The ester **8** was converted to the allylic bromide **10**. Alkylation of the oxazinone **1** with **10** proceeded with high diastereoselectivity. Ethanolysis, hydrogenation of the double bond with diimide, removal of the chiral auxiliary, and hydrolysis gave L-[4-^{13}C]lysine (**2**). Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: L-[4-^{13}C]lysine; labeled amino acid; chiral glycine equivalent; oxazinone

Introduction

Amino acids are the fundamental constituents of proteins and the biosynthetic starting materials of a large number of biologically important primary and secondary metabolites. The use of stable isotope-labeled amino acids has became important for the diagnosis of disease, the study of the biosynthesis of natural products and other biological studies.[1–3] We have reported asymmetric syntheses of L-[3-^{13}C]phenylalanine, L-[3-^{13}C]tyrosine,[4] L-[2-^{13}C]aspartic acid[5] and L-[3-^{13}C]alanine[6] from Dellaria's oxazinone **1**[7] or its ^{13}C-labeled form as a chiral optically active glycine equivalent. This method is also applicable to the synthesis of various amino acids with specific labeling at other positions. In this paper, we describe an asymmetric synthesis of L-[4-^{13}C]lysine (**2**).[8–14]

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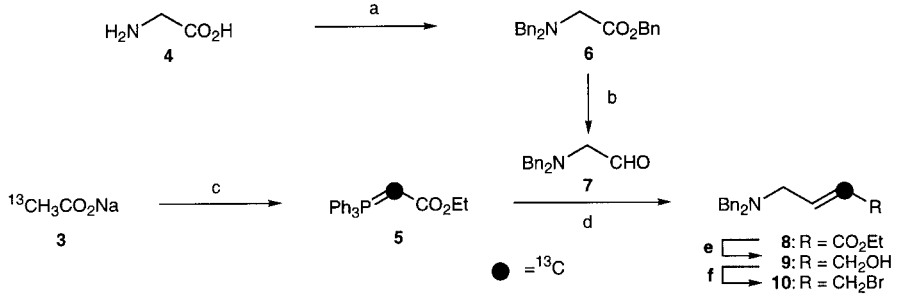
In our synthetic plan, the carbons of lysine originate from two molecules of sodium acetate and one molecule of glycine, both of which are commercially available as ^{13}C-labeled compounds, suitable for the synthesis of multiply and specifically labeled lysine.

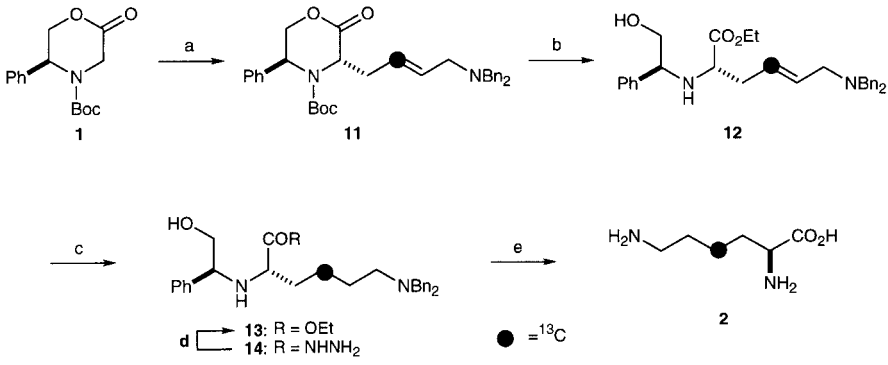
Results and discussion

The C3-C6 unit of lysine was prepared from sodium [2-^{13}C]acetate (**3**) and glycine (**4**) (Scheme 1). Phosphonium ylide **5** was derived from sodium [2-^{13}C]acetate (**3**) via ethyl bromo[2-^{13}C]acetate[5,15] by a usual method in 71% yield from **3**.[16,17] Benzylation of glycine with 3 eq. of benzyl bromide afforded *N*,*N*-dibenzylglycine benzyl ester (**6**) in 96% yield. Conversion to the aldehyde **7** was performed sequentially by $LiAlH_4$ reduction to 2-(dibenzylamino)ethanol and Swern oxidation of the hydroxyl group, in 85% yield.[18] The aldehyde **7** was immediately used in the next step without purification. Wittig reaction of the ^{13}C-labeled phosphonium ylide **5** and **7** gave the α,β-unsaturated ester **8** in 94% yield from **5**. DIBAL reduction of the ester **8** and substitution of the resulting alcohol **9** with LiBr, methanesulfonyl chloride and triethylamine gave the ^{13}C-labeled allylic bromide **10** in 62% yield from **8**.

Alkylation of Dellaria's oxazinone **1** (corresponding to the C1-C2 unit of lysine), which could be prepared in ^{13}C-labeled form, if required, by modifying our reported method from sodium acetate,[5] with the ^{13}C-labeled allylic bromide **10** was performed by treatment of **1** with lithium *bis*(trimethylsilyl) amide (LHMDS) in THF:DME = 5:1, followed by addition of HMPA and **10** to the resulting enolate of **1** at −40°C to give the allylic oxazinone **11** with high diastereoselectivity, *syn*:*anti* = 1:16 (Scheme 2). The ratio was enhanced to 99% de., as determined by HPLC analysis, by crystallization from ethyl acetate–hexane in 86% yield based on **10**. Ethanolysis of the oxazinone ring and removal of the Boc group of **11** were simultaneously carried out by



Scheme 1. ***Reagents and conditions*****: (a) BnCl (3 eq), K_2CO_3, (1.7 eq.), 96%; (b) (i) $LiAlH_4$, 85%, (ii) DMSO, $(COCl)_2$, Et_3N, quant.; (c) (i) PhCOBr, $PhCO_2H$, (ii) Br_2, (iii) EtOH, (iv) Ph_3P, (v) NaOH, 71% from 3; (d) benzene, 94% from 5; (e) DIBAL, 87%; (f) LiBr, MsCl, Et_3N, 71%**



Scheme 2. ***Reagents and conditions*****: (a) LHMDS, then 10 HMPA, −40°C, 86% based on 10; (b) sat. HCl/EtOH, 93%; (c) $KO_2CN = NCO_2K$, AcOH, twice, 13 of 70% and 14 of 30%; (d) sat. HCl/EtOH, 73%; (e) (i) H_2, $Pd(OH)_2$, (ii) 6 M HCl, 51% from 13**

heating at 60°C in ethanol-saturated HCl gas to give **12** in 93% yield. Hydrogenolysis of the three benzyl groups, including the α-*N*-alkyl chain of **12**, and hydrogenation of the double bond proceeded simultaneously on various catalysts, such as Pd–C, $Pd(OH)_2$, PtO_2, and Wilkinson's catalyst, but under all conditions, the unexpected product, ethyl 2-amino[4-^{13}C]hexanote was mainly obtained. Therefore, the double bond alone was hydrogenated initially. Hydrogenation of **12** by treatment with diimide afforded the desired **13**,[19] but the reaction was partial and the starting material **12** was also recovered (**12**:**13** = *ca.* 1:2). The crude mixture of **12** and **13** was hydrogenated once again with diimide. The reaction went to completion, affording **13** and its hydrazide **14**, which was formed from the ester **13** and hydrazine generated from diimide, in 70 and 30% yields, respectively. The hydrazide **14** was readily converted to the ethyl ester **13** with saturated (sat.) HCl in ethanol at 60°C for 6 h in 73% yield. Hydrogenolysis of the three benzyl groups with $Pd(OH)_2$ proceeded cleanly at 60°C in ethanol to give lysine ethyl ester. Finally, hydrolysis of lysine ethyl ester gave L-[4-^{13}C]lysine (**2**) in 51% yield from **13**.

The ^{13}C-NMR spectrum of **2** showed the enriched signal at 24.2 ppm, assigned to the 4-position of lysine. The optical purity was 92% ee and the absolute configuration was L, as determined by HPLC analysis using a chiral column.[20]

In summary, we have synthesized L-lysine (**2**) ^{13}C-labeled at the 4-position, which is the most difficult position in lysine to label specifically, from sodium [2-^{13}C]acetate (**3**) by using diastereoselective alkylation of Dellaria's oxazinone **1**. Other specifically labeled ^{13}C-isotopomers should be similarly obtainable from the appropriate commercially available sodium ^{13}C-acetate and ^{13}C-glycine.

Experimental

Sodium [2-^{13}C]acetate (99 at% ^{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 Varian Gemini-2000 (300 MHz) Fourier-transform spectrometer. The chemical shifts are 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 for ^{1}H-NMR and relative to $CDCl_3$ at 77.0 ppm or TSP at 0 ppm in D_2O for ^{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-700 double-focusing spectrometer. HPLC analysis was carried out on a JASCO 800 Series HPLC system with a JASCO 875-UV detector. HPLC-CD analysis for determination of the optical purity of lysine was performed at 22°C by using a Crown Pak CR(-) column (150 mm × 4 mm i.d.), purchased from Daicel, and pH 1.0 $HClO_4$ as the eluent. Other conditions were as described in a previous report.[20]

*Ethyl 2-(triphenylphosphoranylidene)[2-^{13}C]acetate (**5**)*

Bromo[2-^{13}C]acetyl bromide was prepared from sodium [2-^{13}C]acetate (**3**, 4.82 g) by using the method previously reported.[5,15] To the crude bromo-[2-^{13}C]acetyl bromide was added slowly dropwise dry ethanol (7.0 ml, 121.0 mmol), and the mixture was stirred overnight at room temperature (rt). The mixture was extracted three times with benzene (total 66 ml), then the combined organic layers were washed with sat. NaCl, dried over $MgSO_4$, and filtered. Triphenylphosphine (15.9 g, 61.0 mmol) was added to the solution. The mixture was stirred overnight at rt. The resulting precipitate was collected by filtration, and washed with benzene. The precipitate was dissolved in water (54 ml), and benzene (30 ml) was added. Then 3 M NaOH (8.3 ml, 24.9 mmol) was slowly added dropwise to the mixture at rt with vigorous stirring. The organic layer was separated, and the aqueous layer was extracted three times with benzene. The combined organic layers were washed with sat. NaCl, dried over $MgSO_4$, and evaporated. The crude product was crystallized from $CHCl_3$-ether to give **5** (14.5 g) in 71% yield. mp. 124.9–126.3°C. ^{1}H-NMR ($CDCl_3$) δ: 0.88–1.35 (br, 3H), 2.45–3.44 (br-d, $J_{C\text{-}H} = 148$ Hz, 1H), 3.97 (br, 2H), 7.51–7.69 (m, 15H). ^{13}C-NMR ($CDCl_3$) δ: 30.2 (d, $J_{C\text{-}P} = 126.0$ Hz). IR (KBr) cm^{-1}: 3057, 2977, 2901, 1605, 1483, 1437, 1368, 1323, 1300, 1121, 1106, 1060, 879, 755, 696, 520, 507. HR-MS Calcd for C_{21} $^{13}CH_{21}O_2P$: m/z 349.1313. Found: m/z 349.1305 (M^+). EI-MS m/z (%): 349 (M^+, 19), 302 (100), 277 (21).

*Ethyl (E)-4-dibenzylamino-2-[2-^{13}C]butenoate (**8**)*

Benzylation of glycine (**4**, 5.00 g, 66.6 mmol) with 3.0 eq. of benzyl bromide and 1.7 eq. of K_2CO_3 in water at 100°C for 15 h gave the ester **6** (22.18 g) in 96% yield. Reduction of the ester **6** (22.2 g, 64.2 mmol) with $LiAlH_4$ in THF at rt for 3 h gave 2-(dibenzylamino)ethanol (13.2 g) in 85% yield. Swern oxidation of this alcohol (5.03 g, 20.8 mmol) with 7 eq. of DMSO, 5 eq. of oxalyl chloride and 12 eq. of triethylamine gave 7.12 g of crude *N*,*N*-dibenzylglycinal (**7**).[18]

To a solution of the resulting **7** (corresponding to 20.8 mmol) in benzene (70 ml) was added ^{13}C-labeled phosphonium ylide **5** (6.55 g, 17.8 mmol). The mixture was stirred for 13 h at rt, then evaporated to $\frac{1}{3}$ volume. A mixture of hexane and ether (4:1, 300 ml) was added. The precipitated phosphine oxide was filtered off, and the filtrate was evaporated. The crude product was purified by column chromatography on silica gel (hexane:ethyl acetate = 7:1) to give **8** (5.45 g) in 94% yield from **5**. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, J = 7.1 Hz, 3H), 3.19 (dt, J = 1.6, 5.8 Hz, $^3J_{C\text{-}H}$ = 5.8 Hz, 2H), 3.59 (s, 4H), 4.19 (q, J = 7.1 Hz, 2H), 6.08 (ddt, $J_{C\text{-}H}$ = 162.6 Hz, J = 15.7, 16 Hz, 1H), 7.00 (ddt, $^2J_{C\text{-}H}$ = 2.2 Hz, J = 15.7, 5.8 Hz, 1H), 7.20–7.40 (m, 10H). ^{13}C-NMR ($CDCl_3$) δ: 122.7. IR (neat) cm^{-1}: 3029, 2981, 2931, 2799, 1718, 1495, 1368, 1304, 1264, 1178, 1029, 747, 699. HR-MS Calcd for C_{19} $^{13}CH_{23}NO_2$: m/z 310.1762. Found: m/z 310.1763 (M^+). EI-MS m/z (%): 310 (M^+, 6), 281 (8), 265 (6), 233 (12), 219 (92), 210 (10), 91 (100).

*(E)-4-Dibenzylamino-2-[2-^{13}C]buten-1-ol (**9**)*

To a stirred solution of the ester **8** (4.80 g, 15.5 mmol) in dry CH_2Cl_2 (40 ml) was added dropwise a 1 M solution of DIBAL in toluene (43.4 ml, 43.4 mmol) over 1 h at −78°C, and the mixture was stirred for 2 h. The reaction was quenched with MeOH, and a large amount of ether and a proper amount of sat. NaCl were added to precipitate aluminous salt. The precipitate was filtered off, and the filtrate was evaporated. The crude product was chromatographed on silica gel to give the alcohol **9** (3.53 g) in 87% yield. ^{1}H-NMR ($CDCl_3$) δ: 3.07 (t, $^3J_{C\text{-}H}$ = 5.8 Hz, J = 5.8 Hz, 2H), 3.58 (s, 4H), 4.10 (m, 2H), 5.77 (m, 1H), 5.79 (ddt, $J_{C\text{-}H}$ = 154.4 Hz, J = 15.4, 5.8 Hz, 1H), 7.20–7.39 (m, 10H). ^{13}C-NMR ($CDCl_3$) δ: 131.7. IR (neat) cm^{-1}: 3342, 3027, 2796, 1494, 1454, 1366, 1124, 1074, 1028, 971, 746, 698. HR-MS Calcd for C_{17} $^{13}CH_{21}NO$: m/z 268.1657. Found: m/z: 268.1665 (M^+). EI-MS m/z (%): 268 (M^+, 9), 210 (20), 197 (25), 191 (19), 106 (34), 91 (100).

*(E)-Dibenzyl(4-bromo-2-[3-^{13}C]butenyl)amine (**10**)*

A mixture of alcohol **9** (3.53 g, 13.2 mmol) and LiBr (17.2 g, 198.3 mmol) was dissolved in dry THF (73 ml) at 0°C, and triethylamine (9.2 ml, 66.1 mmol) was

added. Methanesulfonyl chloride was added dropwise to the mixture at −78°C, and the whole was slowly warmed to −40°C over 2 h. The mixture was stirred for 1 h at that temperature, and then allowed to warm to 0°C. The mixture was poured into sat. $NaHCO_3$, and extracted five times with ether. The combined organic layers were washed with sat. NaCl, dried over $MgSO_4$, and evaporated at 0°C. The residue was rapidly chromatographed on silica gel (hexane:ether = 10:1) to give the bromide **10** (3.12 g) in 71% yield. ^{1}H-NMR ($CDCl_3$,) δ: 3.07 (t, $^3J_{C\text{-}H}$ = 6.0 Hz, J = 6.0 Hz, 2H), 3.57 (s, 4H), 3.95 (ddd, $^2J_{C\text{-}H}$ = 4.1 Hz, J = 7.7, 0.5 Hz, 2H), 5.85 (m, 1H), 5.86 (ddt, $J_{C\text{-}H}$ = 160.2 Hz, J = 15.1, 7.4 Hz, 1H), 7.20–7.39 (m, 10H). ^{13}C-NMR ($CDCl_3$) δ: 129.0.

*(E)-(3S,5S)-2,3,5,6-Tetrahydro-3-(4-dibenzylamino-[2-^{13}C]butenyl)-5-phenyl-N-(tert-butyloxycarbonyl)-4H-1,4-oxazin-2-one (**11**)*

LHMDS was prepared from BuLi (1.58 M in hexane solution, 8.6 ml, 13.6 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (3.1 ml, 14.7 mmol) in a mixture of THF (8 ml) and DME (4 ml) at 0°C under nitrogen. To a solution of oxazinone **1** (3.13 g, 11.3 mmol) in a mixture of THF (10 ml) and DME (5 ml) was added dropwise the LHMDS solution though a cannula at −40°C under nitrogen. The flask was washed with THF (6 ml), and the solution was similarly added to the LHMDS solution. The mixture was stirred for 30 min, then HMPA (2.62 ml, 15.1 mmol) was added. This mixture was stirred for 10 min, then a solution of the bromide **10** (3.12 g, 8.44 mmol) in THF (16 ml) and DME (3 ml) was added dropwise over 20 min. The reaction mixture was stirred at −40°C overnight. The reaction was quenched with sat. NH_4Cl, and the mixture was extracted five times with ether. The combined organic layers were washed with sat. NaCl, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (hexane:ethyl acetate = 4:1). Pure **11** (4.28 g, 86% yield) was obtained by crystallization from hexane–ethyl acetate. mp. 142.4–143.3°C. ^{1}H-NMR ($CDCl_3$, 55°C) δ: 1.26 (brs, 9H), 2.74 (m, 2H), 3.06 (t, J = 5.8 Hz, 2H), 3.58 (s, 4H), 4.31 (m, 1H), 4.71 (dd, J = 11.5, 3.0 Hz, 1H), 4.71–5.10 (m, 2H), 5.68 (m, $J_{C\text{-}H}$ = 154.4 Hz, 1H), 5.70 (m, 1H), 7.06 (m, 2H), 7.18–7.37 (m, 13H). ^{13}C-NMR ($CDCl_3$, 55°C) δ: 126.6. IR (KBr) cm^{-1}: 2977, 2923, 2797, 1740, 1699, 1453, 1359, 1297, 1221, 1169, 1123, 1075, 746, 698. HR-MS Calcd for C_{32} $^{13}CH_{38}N_2O_4$: m/z 527.2865. Found: m/z 527.2874 (M^+). EI-MS m/z (%): 527 (M^+, 31), 470 (10), 336 (16), 251 (49), 237 (13), 210 (14), 196 (44), 106 (25), 91 (100). $[\alpha]_D^{20}$ = +114.0° (*c*. 1.01, $CHCl_3$).

*Ethyl (2S)-6-dibenzylamino-2-[(S)-2-hydroxy-1-phenylethylamino)]-4-[4-^{13}C] hexenoate (**12**)*

A solution of the oxazinone **11** (2.02 g, 3.83 mmol) in sat. HCl in ethanol (40 ml) was stirred at 60°C for 6 h. The mixture was evaporated, and the

residue was made basic with sat. $NaHCO_3$. The mixture was extracted four times with ethyl acetate, and the combined organic layers were washed with sat. NaCl, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel to give **12** (1.68 g) in 93% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (t, $J = 7.1$ Hz, 3H), 2.41 (q, $^2J_{C\text{-}H} = 6.3$ Hz, $J = 6.3$ Hz, 2H), 3.00 (t, $^3J_{C\text{-}H} = 6.0$ Hz, $J = 6.0$ Hz, 2H), 3.31 (dt, $^3J_{C\text{-}H} = 4.7$ Hz, $J = 6.0$ Hz, 1H), 3.53 (dd, $J = 11.0$, 8.2 Hz, 1H), 3.54 (s, 4H), 3.65 (dd, $J = 11.0$, 4.7 Hz, 1H), 3.75 (dd, $J = 8.2$, 4.7 Hz, 1H), 3.87 (q, $J = 7.1$ Hz, 2H), 4.31 (m, 1H), 5.57 (m, $J_{C\text{-}H} = 152.7$ Hz, 1H), 5.63 (m, 1H), 7.19–7.39 (m, 15H). ^{13}C-NMR ($CDCl_3$) δ: 128.0. IR (neat) cm^{-1}: 3440, 3332, 3028, 2925, 2796, 1734, 1494, 1454, 1371, 1196, 1028, 972, 748, 699. HR-MS Calcd for $C_{29}{}^{13}CH_{36}N_2O_3$: m/z 473.2759. Found: m/z 473.2766 (M^+). EI-MS m/z (%): 473 (M^+, 0.2), 442 (24), 382 (23), 336 (18), 278 (16), 251 (17), 222 (21), 203 (33), 196 (40), 121 (18), 106 (29), 91 (100). $[\alpha]_D^{20} = +31.3^\circ$ (*c*. 1.03, $CHCl_3$).

*Ethyl (2S)-6-dibenzylamino-2-[(S)-2-hydroxy-1-phenylethylamino)]-4-[4-^{13}C] hexanoate (**13**)*

To a mechanically stirred mixture of **12** (1.68 g, 3.54 mmol) and dipotassium azodicarboxylate (48.8 g, 248 mmol) in dry acetonitrile (350 ml) was added dropwise acetic acid (28.3 ml, 495 mmol) at 70°C over 8 h, and the mixture was stirred for an additional 3 h. The mixture was made basic with sat. $NaHCO_3$, and filtered through Celite, then the filtrate was evaporated. Sat. $NaHCO_3$ was added to the residue, and the mixture was extracted five times with ethyl acetate. The combined organic layers were washed with sat. NaCl, dried over $MgSO_4$, and evaporated. The crude product was treated once again under these conditions for diimide reduction. The resulting products were separated by chromatography on silica gel (hexane:ethyl acetate = 1:1) to give **13** (1.17 g) and **14** (548 mg) in 70 and 30% yields, respectively. **13**: ^{1}H-NMR ($CDCl_3$) δ: 1.09 (t, $J = 7.1$ Hz, 3H), 1.05–1.60 (m, 6H), 2.39 (m, 2H), 3.18 (m, 1H), 3.53 (s, 4H), 3.45 (dd, $J = 12.1$, 9.3 Hz, 1H), 3.63–3.71 (m, 2H), 3.87 (q, $J = 7.1$ Hz, 2H), 7.19–7.38 (m, 15H). ^{13}C-NMR ($CDCl_3$) δ: 23.3. IR (neat) cm^{-1}: 3339, 3062, 2937, 2860, 2796, 1731, 1494, 1454, 1372, 1191, 1028, 748, 699. HR-MS Calcd for $C_{29}{}^{13}CH_{38}N_2O_3$: m/z 475.2910. Found: m/z 475.2913 (M^+). EI-MS m/z (%): 475 (M^+, 0.3), 429 (27), 384 (23), 338 (52), 210 (100), 104 (9), 91 (88). $[\alpha]_D^{20} = +30.5^\circ$ (*c*. 1.03, $CHCl_3$). **14**: ^{1}H-NMR ($CDCl_3$) δ: 1.05–1.80 (m, 6H), 2.41 (m, 2H), 3.02 (m, 1H), 3.53 (d, $J = 13.6$ Hz, 2H), 3.55 (d, $J = 13.6$ Hz, 2H), 3.60 (dd, $J = 11.3$, 9.3 Hz, 1H), 3.64–3.74 (m, 2H), 7.20–7.39 (m, 15H), 7.82 (s, 1H). ^{13}C-NMR ($CDCl_3$) δ: 23.2. IR (neat) cm^{-1}: 3321, 3028, 2934, 2858, 2797, 1656, 1494, 1454, 1366, 1127, 1058, 1028, 750, 700. EI-MS m/z (%): 461 (M^+, 0.7), 430 (8), 402 (16), 370 (48), 355 (23), 338 (12), 310 (11), 210 (71), 106 (11), 91 (100).

*L-[4-^{13}C]lysine dihydrochloride (**2**)*

Benzylamine **13** (1.44 g, 3.03 mmol) was dissolved in ethanol (20 ml), and 20% $Pd(HO)_2$ on carbon (4.25 g, 6.05 mmol) was added. The mixture was stirred at 60°C for 39 h under a hydrogen atmosphere. The catalyst was removed by filtration through Celite, and the filtrate was evaporated. The residue was dissolved in water and washed three times with ether. The combined organic layers were extracted three times with water. All aqueous layers were combined and evaporated at 30°C to give a mixture (390 mg) of lysine ethyl ester and small amounts of lysine, partially hydrolyzed by work-up, as the residue. The residue (314 mg) was dissolved in 6 M HCl (30 ml). The mixture was heated at 85°C for 8 h, then evaporated to give crude **2** (579 mg). Crystallization from ethanol-ether (390 mg) gave pure L-[4-^{13}C]lysine dihydrochloride (**2**) in 51% yield from **13**. mp. 184.3–185.4°C. HPLC-CD: Rt = 8.6 min (trace D-form gave Rt = 11.0 min). ^{1}H-NMR (D_2O) δ: 1.10–1.90 (m, 6H), 2.89 (m, 2H), 3.84 (m, 1H). ^{13}C-NMR (D_2O) δ: 24.2. IR (KBr) cm^{-1}: 3423, 3100–2300 (br), 1736, 1618, 1509, 1501, 1212, 1129, 998, 821, 735. HR-FAB-MS (glycerol) Calcd for $C_5\,{}^{13}CH_{14}N_2O_2$: m/z 148.0586. Found: m/z 148.1175 (MH^+). CD (pH 1.0 $HClO_4$) λ nm ($\Delta\varepsilon$): 196 (+4.25), 211 (+2.67).

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