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ORIGINAL ARTICLE

Synthesis of 4-thia-[6-^{13}C]lysine from [2-^{13}C]glycine: access to site-directed isotopomers of 2-aminoethanol, 2-bromoethylamine and 4-thialysine

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Abstract 4-Thialysine (*S*-(2-aminoethyl)-L-cysteine) is an analog of lysine. It has been used as an alternative substrate for lysine in enzymatic reactions. Site-directed isotopomers are often needed for elucidation of mechanism of reactions. 4-Thialysine can be synthesized by reacting cysteine with 2-bromoethylamine, an important reagent in chemical-modification rescue (CMR) of proteins. Here, we present the synthesis of 4-thia-[6-^{13}C]lysine, one of the isotopomers of 4-thialysine, from commercially available starting material [2-^{13}C]glycine via formation of five intermediates including 2-amino[2-^{13}C]ethanol and 2-bromo[1-^{13}C]ethylamine. The compounds were characterized using various spectroscopic techniques. Moreover, we discuss that our strategy would provide access to site-directed isotopomers of 2-aminoethanol, 2-bromoethylamine and 4-thialysine. Biological activity of 4-thia-[6-^{13}C]lysine was tested in the enzymatic reaction of lysine 5,6-aminomutase.

Keywords Amino acids · Carbon labels · Site-directed isotopomers · 4-Thialysine · 2-Bromoethylamine · 2-Aminoethanol

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Introduction

Isotopomers have been extensively used in the study of mechanism and structure determination. Labeling with radioisotopes finds extensive application for tracking molecule or molecular fragment in various chemical and biochemical processes. It has been widely used in enzymology and molecular biology. Nevertheless, labeling with stable isotopes is also of great importance (Lloyd-Jones and Munoz 2007). Labeling proteins with stable isotopes is a common practice to study protein structure, dynamics and quantification using various spectroscopic techniques such as mass (Tao and Aebersold 2003), vibrational (Decatur 2006; Ludlam et al. 1995) and magnetic resonance spectroscopy (Macnaughtan et al. 2005). Isotopic substitution, which usually results in substitution of nuclei with nuclei having different spin multiplicity, has been extensively used to simplify the spectra obtained from magnetic resonance spectroscopy to get more information. Recently, stereo-array isotope labeling approach, together with advanced nuclear magnetic resonance (NMR), was employed for determination of protein structure and hydrogen exchange rates of hydroxyl groups of tyrosine residues in proteins (Kainosho et al. 2006; Takeda et al. 2009). Electron paramagnetic resonance (EPR) combined with specific isotope labeling has been instrumental to study the mechanism of reactions involving radicals (Frey et al. 2006). For example, this approach has successfully been employed to elucidate the mechanism of the reaction involving lysine aminomutases (Behshad et al. 2006; Frey et al. 2006; Lees et al. 2006; Wu et al. 1995). The synthesis of site-specific isotopomers of lysine was reported earlier (Raap et al. 1995; Siebum et al. 2004a). 4-Thialysine (*S*-(2-aminoethyl)-L-cysteine) in which 4-methylene group of lysine is replaced by a sulfur atom is a very effective analog of lysine and has been

widely used to mimic the chemistry involving lysine. For instance, it was used in the study of feedback inhibition of α-aminoadipate reductase, a key enzyme for biosynthesis of lysine in yeast (Suvarna et al. 1998). In mammalian cells and human parasites of the genus *Leishmania*, 4-thialysine is the substrate for the enzyme thialysine N^{ε}-acetyltransferase (Coleman et al. 2004; Luersen 2005). 4-Thialysine undergoes metabolism to form cyclic ketimine derivatives found in mammalian tissues including brain and as urinary metabolites (Coleman et al. 2004). These metabolites can undergo further reactions to form compounds which have been postulated to serve neurochemical roles or to have antioxidant properties. Interestingly, possible application of 4-thialysine as potential chemotherapeutic agent for cancer has been proposed on the basis of its observed cytotoxicity toward human acute leukemia Jurkat T cells (Jun et al. 2003). Recently, 4-thialysine has been used (Maity et al. 2009; Tang et al. 2009) to elucidate the mechanism of lysine 5,6-aminomutase, an adenosylcobalamin (AdoCbl) and pyridoxal-5′-phosphate (PLP) dependent enzyme which catalyzes reversible 1,2-shift of the ε-amino group of DL-lysine or L-β-lysine into 2,5- or 3,5-diaminohexanoic acid. The reaction is proposed to follow a radical mechanism involving substrate–PLP radical ($S^{\cdot}$), a cyclic aziridinylcarbinyl–PLP radical ($I^{\cdot}$) and product–PLP radical ($P^{\cdot}$) (Scheme 1).

Uniform and partial labeling of 4-thialysine could not unambiguously identify the radical intermediate. The site-directed labeling at carbon-5 and carbon-6 of 4-thialysine would help to identify the radical intermediate. Although some of the isotopomers have previously been synthesized, the report of the synthesis of site-directed isotopomers at δ and ε is absent. Several labeled 4-thialysine were prepared by reacting labeled cysteine with 2-bromoethylamine following the procedure which was first reported by Cavallini et al. (1955). However, detailed characterizations of the compounds were not reported. 2-Bromoethylamine is an important reagent in chemical-modification rescue (CMR) of proteins (Gloss and Kirsch 1995a, b; Hopkins et al. 2002). In this process active site mutation of lysine to cysteine, followed by aminoethylation of cysteine residue to 4-thialysine is correlated with loss and restoration of activity. CMR has been used to investigate the role of active site lysine residues in enzymatic catalysis. Recently, isotopomers of 2-bromoethylamine have been utilized in the study of chemical rescue by electrospray ionization Fourier transform mass spectrometry (Hopkins et al. 2002) and NMR (Hopkins et al. 2005). 2-Bromoethylamine can be synthesized (Cortese 1943) from 2-aminoethanol which is a synthon for synthesis of many important molecules including natural products. So, there is a need to develop a strategy to synthesize all possible isotopomers of 4-thialysine, ethanolamine and 2-bromoethylamine. In this report, we describe the synthesis of 4-thia-[6-^{13}C]lysine starting from [2-^{13}C]glycine through intermediate formation of 2-amino[2-^{13}C]ethanol and 2-bromo-[1-^{13}C]ethylamine. Additionally, we discuss the strategy to access site-directed isotopomers of 4-thialysine, 2-aminoethanol and 2-bromoethylamine.

Materials and methods

Materials

All commercially available compounds were purchased from Sigma–Aldrich, Across or Fluka. All chemicals were used without further purification. [2-^{13}C]glycine (>98% isotope-enriched) was purchased from Cambridge Isotope Laboratories. HPLC-grade solvents were used.

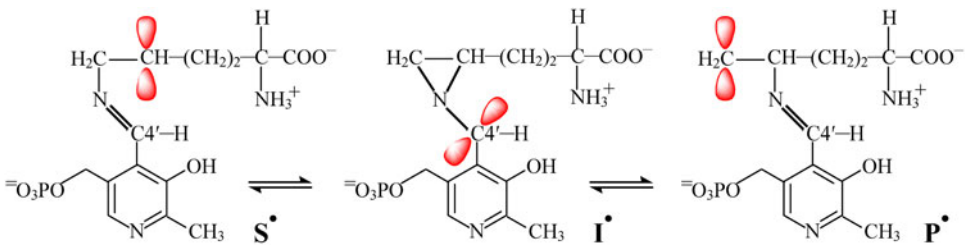
Methods

^{1}H and ^{13}C NMR spectra were recorded using a Bruker Spectrospin NMR spectrometer. NMR chemical shifts were referenced to TMS ($CDCl_3$) and TSP (D_2O). Infrared spectra were acquired by Perkin–Elmer spectrophotometer. Only the major bands in IR spectra are reported. Optical rotations were measured using a Jasco P-1010 polarimeter. Elemental analyses were performed on a Flash EA 1112 elemental analyzer. Mass spectra were recorded on Bruker Daltonic autoflex MALDI-TOF mass spectrometer. The values of *m/z* were calculated using ChemDraw Ultra software.

*[2-^{13}C]Glycine ethyl ester hydrochloride (**1**)*

A suspension of [2-^{13}C]glycine (2.00 g, 26.3 mmol) in ethanol (160 mL) was taken to ice bath. To this suspension was added $SOCl_2$ (13.5 mL, 185.8 mmol) over a period of

Scheme 1 Radical intermediates in the reaction of D-lysine and 5,6-LAM



30 min. The reaction mixture was warmed to room temperature and stirred for a further 12 h. The solvent was evaporated under reduced pressure to produce the product **1** as white needle-like crystals (yield = 3.65 g, 99%). Found: C, 32.08; H, 6.89; N, 9.99. Anal. Calcd. for $C_3^{13}CH_{10}ClNO_2$: C, 34.88; H, 7.17; N, 9.96. IR (KBr, cm^{-1}): 2,800–3,100 (br), 1,745, 1,576, 1,549, 1,501, 1,457, 1,423, 1,404, 1,384, 1,250, 1,040, 900, 854. ^{1}H NMR (400 MHz, D_2O): δ 1.14 (t, 3H, $^2J_{HH} = 7.2$ Hz), 3.75 (d, 2H, $^1J_{CH} = 145.8$ Hz), 4.14 (q, 2H, $^2J_{HH} = 7.2$ Hz). ^{13}C NMR (100 MHz, D_2O) δ 13.13, 40.13, 63.24, 168.12 (d, $^1J_{CC} = 62.0$ Hz). MALDI-TOF MS: $m/z = 104.779$ (calcd. for $[M–Cl]^+$ 105.075).

*Ethyl 2-(tert-butoxycarbonylamino)[2-^{13}C]acetate (**2**)*

Triethylamine (4.5 mL, 32.3 mmol) was added slowly to a solution of **1** (1.80 g, 12.8 mmol) in methanol (11 mL) at 4°C. To this mixture di-*tert*-butyl dicarbonate (3.0 mL, 13.1 mmol) was added slowly. The resultant mixture was stirred under nitrogen for 24 h. The solvent was evaporated in vacuo. Water (10 mL) and EtOAc (10 mL) were added to the above residue, acidified with 1 N HCl solution (pH 5–6). The organic layer was separated and the aqueous layer was extracted with EtOAc (10 mL). The combined EtOAc extract was washed with 1 N HCl, 5% $NaHCO_3$ and brine, respectively. The organic layer was then dried over anhydrous $MgSO_4$ and concentrated to afford the product **2** as colorless oil. (yield = 2.52 g, 96%). Found: C, 52.23; H, 8.56; N, 7.41. Anal. Calcd. for $C_8^{13}CH_{17}NO_4$: C, 53.42; H, 8.39; N, 6.86. IR (KBr, cm^{-1}): 3,375, 2,980, 2,935, 1,753, 1,720, 1,702 (sh), 1,519, 1,455, 1,392, 1,368, 1,350, 1,283, 1,251, 1,203, 1,169, 1,052, 1,029, 945, 863, 784, 764, 530–620 (br). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, 3H, $^3J_{HH} = 7.1$ Hz), 1.43 (s, 9H), 3.88 (dt, 2H, $^1J_{CH} = 145.7$ Hz, $^3J_{HH} = 5.5$ Hz), 4.19 (q, 2H, $^3J_{HH} = 7.2$ Hz), 5.01(br, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.16, 28.31, 42.29, 43.86, 61.34, 79.97, 155.72.

*2-(tert-Butoxycarbonylamino)[2-^{13}C]ethanol (**3**)*

To a solution of **2** (2.50 g, 12.3 mmol) in THF (13 mL) were added sodium borohydride (1.39 g, 36.6 mmol), lithium chloride (1.55 g, 36.6 mmol) and methanol (25 mL). The mixture was stirred at room temperature for 28 h. 15 mL of 5% citric acid was added to the reaction mixture and extracted with CH_2Cl_2. The organic extract was washed with 5% $NaHCO_3$ and brine, respectively, dried over anhydrous $MgSO_4$ and concentrated to afford the crude product as colorless oil. The crude product was used for the next step without further purification. (yield = 1.46 g, 73%). Found: C, 49.08; H, 9.49; N, 9.03. Anal. Calcd. for $C_6^{13}CH_{15}NO_3$: C, 52.45; H, 9.32; N, 8.64. IR (KBr, cm^{-1}): 3,352, 2,978, 2,933, 2,877, 1,690, 1,525, 1,456, 1,393, 1,367, 1,277, 1,252, 1,172, 1,140 (sh) 1,063, 864, 781, 756, 720–580 (br). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H), 2.40 (bs, 1H), 3.28 (dt, 2H, $^1J_{CH} = 137.1$ Hz, $^3J_{HH} = 4.6$ Hz), 3.70 (t, 2H, $^3J_{HH} = 5.3$ Hz), 5.01 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.38, 43.21, 62.65 (d, $^1J_{CC} = 42.0$ Hz), 79.74, 156.89.

*2-Amino[2-^{13}C]ethanol hydrochloride (**4**)*

HCl gas was bubbled through a solution of **3** (1.38 g, 8.5 mmol) in CH_2Cl_2 for 30 min. The completion of reaction was indicated by the formation of large amount of solid formation. The solid was filtered under vacuum and washed with CH_2Cl_2 (10 mL) to produce the product **4** as white solid. (yield = 0.82 g, 98%). Found: C, 22.38; H, 8.42; N, 12.90. Anal. Calcd for $C^{13}CH_8ClNO$: C, 25.39; H, 8.18; N, 14.21. IR (KBr, cm^{-1}): 3,848, 3,741, 3,412, 3,070, 1,624, 1,502, 1,316, 1,262, 1,105, 1,056, 999, 863, 814, 765, 708, 652, 615, 593, 554, 499. ^{1}H NMR (400 MHz, D_2O): δ 2.99 (dt, 2H, $^1J_{CH} = 144.1$ Hz, $^3J_{HH} = 5.4$ Hz), 3.67 (t, 2H, $^3J_{HH} = 5.2$ Hz). ^{13}C NMR (100 MHz, D_2O) δ 41.20, 57.51 (d, $^1J_{CC} = 38.0$ Hz). MALDI-TOF MS: $m/z = 62.747$ (calcd. for $[M–Cl]^+$ 63.064).

*2-Bromo[1-^{13}C]ethylamine hydrobromide (**5**)*

Forty-eight percent hydrobromic acid (6.6 mL) was added to **4** (788 mg, 8.0 mmol) in a 10-mL round bottom flask attached to a distillation assembly and heated in an oil bath until 1.5 mL distillate was collected. Then the temperature was lowered and maintained in such a way so that it ceased to distill and merely refluxed. After refluxing for 1 h, 1.4 mL more was distilled and the solution was heated under reflux for 1 h. This procedure was repeated for 1, 0.7, 0.3 and 0.2 mL, respectively. During all these operations, precaution should be taken so that the temperature of the oil bath does not exceed 160°C as the compound decomposes. The last three portions of distillate were distilled under reduced pressure. The reaction mixture was cooled to 70°C and acetone was added and stirred well. After standing overnight at 4°C, it was filtered to obtain the product as white solid which was washed with acetone and air dried. Concentrating the filtrate to syrup, adding acetone and standing overnight at 4°C, a second crop of material was obtained. Compound **5** is hygroscopic. (yield = 1.01 g, 61%). Found: C, 11.58; H, 3.40; N, 6.75. Anal. Calcd. for $C^{13}CH_7Br_2N$: C, 12.15; H, 3.43; N, 6.80. IR (KBr, cm^{-1}): 3,427, 3,100–2,750 (br), 1,930–1,830 (br), 1,580, 1,560, 1,504, 1,483, 1,432, 1,373, 1,320, 1,251, 1,231, 1,088, 1,032, 935, 869, 819, 763, 708, 651, 570, 496. ^{1}H NMR (400 MHz, D_2O): δ 3.15 (t, 1H, $^3J_{HH} = 6.1$ Hz), 3.50–3.57 (m, 3H). ^{13}C NMR (100 MHz, D_2O) δ 27.84

(d, $^{1}J_{CC}$ = 37.0 Hz), 41.00. MALDI-TOF MS: *m/z* 124.711, 126.714 (calcd. for $[M–Br]^{+}$ 124.980, 126.977).

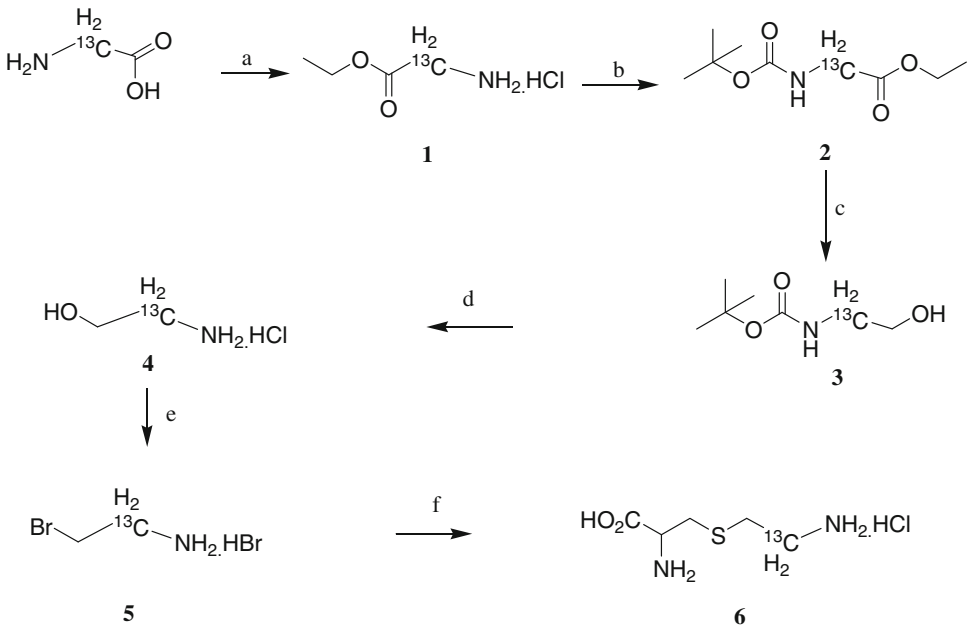
*4-Thia-[6-^{13}C]lysine hydrochloride (**6**)*

KOH (442 mg, 7.8 mmol) and cysteine hydrochloride (368 mg, 2.3 mmol) were taken in a 25-mL Schlenk flask and evacuated and purged with nitrogen. Oxygen-free water (two cycles of freeze–pump–thaw) was added to the flask under nitrogen atmosphere and heated in an oil bath at 70°C. Compound **5** (515 mg, 2.5 mmol) was added slowly with stirring to the solution under nitrogen atmosphere for 10 min. The mixture was kept at 70°C for another 10 min. It was brought to room temperature under nitrogen atmosphere and kept for 7 h. Then it was neutralized with 48% HBr. 2 mL of EtOH added and precipitate appeared. It was dissolved by adding water dropwise. Little EtOH was added to make it little turbid and kept at 4°C for 12 h. Then it was filtered through filter paper (Advantec 5B) and the filtrate was passed through a column of cation exchanger (Dowex 50w × 8 in H^{+} form), washed with water and eluted with 1 N ammonium hydroxide. The fractions which had shown positive ninhydrin test were collected. The collected fractions were evaporated to dryness using rotary evaporator. The oily residue was dissolved in minimum amount of water and neutralized to slight acidity (pH ~6) by conc. HCl. Then EtOH (2 mL) and of acetone (3 mL) was added and kept at 4°C for 12 h. It was filtered to produce the crude product which was recrystallized twice from water, ethanol and acetone mixture to produce needle-like crystals. (yield = 276 mg, 55%). $[\alpha]_{D}^{19} = -3.9°$ (c 1.0, H_2O); Found: C, 29.03; H, 6.39; N, 13.91. Anal. Calcd for $C_4^{13}CH_{13}ClN_2O_2S$: C, 30.27; H, 6.50; N, 13.89. IR (KBr, cm^{-1}): 3,436, 3,200–2,750 (br), 2,676, 2,558, 1,998, 1,634, 1,567, 1,488, 1,461, 1,414, 1,396, 1,385, 1,351, 1,327, 1,313, 1,140, 1,121, 1,089, 1,072, 1,047, 960, 933, 882, 844, 773, 550, 466. ^{1}H NMR (400 MHz, D_2O): δ 2.72–2.77 (2H, m), 2.96 (2H, t, $^{3}J_{HH}$ = 4.3 Hz), 3.09 (2H, dt, $^{1}J_{CH}$ = 145.3 Hz, $^{3}J_{HH}$ = 6.6 Hz), 3.82 (1H, t, $^{3}J_{HH}$ = 5.4 Hz). ^{13}C NMR (100 MHz, D_2O) δ 28.43, 31.51, 38.15, 53.40, 172.60. MALDI-TOF MS: *m/z* = 165.845 (calcd. for $[M–Cl]^{+}$ 166.073).

Results and discussion

In order to synthesize the desired isotopomers of 4-thialysine, we need to design a strategy keeping in mind some factors such as commercial availability and cost effectiveness of corresponding labeled starting material, intermediates that can be isolated easily and reactions with high yield. As we mentioned earlier, the reaction between cysteine and 2-bromoethylamine can be used as the final step of the scheme. Isotopomers of cysteine are commercially available while isotopomers of 2-bromoethylamine are not. However, it can be synthesized easily from 2-aminoethanol (Cortese 1943). Although some of the isotopomers of 2-aminoethanol are commercially available, they are expensive, specially the site-directed ones. On the contrary, it can be synthesized (Babior 1969) from relatively inexpensive starting material, glycine. So, we chose glycine as the starting material. Before starting with the labeled compound, we tried to standardize all the required steps with unlabelled compounds. The synthesis of 2-aminoethanol, following the reported procedure (Babior 1969), by directly reducing the ethyl glycinate with $LiAlH_4$ and continuous extraction for 2 days resulted in poor yield. In the second attempt following another literature report (Verhoeven et al. 2004), protection of amino group by benzaldehyde followed by reduction with $LiAlH_4$ and subsequent reduction with H_2 and 10% Pd/C produced mixture of products in our hands. Moreover, *N*-benzylidene-glycine ethyl ester, obtained by reacting glycine ethyl ester with benzaldehyde, slowly decomposes in air. Then, another attempt by blocking amino group with benzoyl chloride, reduction with $LiAlH_4$ and subsequent reduction with H_2 and Pd/C also produced mixture of products. Therefore, we planned a new strategy that produced satisfactory yields for all the steps. Then, we followed the strategy starting with [2-^{13}C]glycine (Scheme 2).

In this method we synthesized ethyl [2-^{13}C]glycinate (**1**) by reacting thionyl chloride (Stocking et al. 2001) with [2-^{13}C]glycine in ethanol. This method appeared to be better than the esterification with HCl gas as the reaction is incomplete in the latter case if the reaction mixture is not saturated with HCl. Ethyl [2-^{13}C]glycinate was then reacted with di-*tert*-butyl dicarbonate (Boc_2O) (Kim et al. 2007) to get ethyl 2-(*tert*-butoxycarbonylamino)[2-^{13}C]acetate (**2**) which was reduced by $NaBH_4$ to produce 2-(*tert*-butoxycarbonylamino)[2-^{13}C]ethanol (**3**). The reduction with $NaBH_4$ was initially performed in ethanol following the literature procedure (Iizuka et al. 1990). Very little product was obtained after stirring at room temperature. Despite refluxing overnight, the reaction was found to be incomplete. The use of methanol instead of ethanol resulted in almost quantitative yield at room temperature. As ethanol is one of the byproducts of the reaction, the presence of bulk amount of ethanol as solvent probably moves the reaction equilibrium towards left. **3** was converted to 2-amino[2-^{13}C]ethanol hydrochloride (**4**) by reacting with HCl gas. Bromination of **4** with HBr produced 2-bromo[1-^{13}C]ethylamine hydrobromide (**5**). The proton NMR spectrum of **5** shows a triplet and a multiplet instead of the expected single triplet due to coupling from two protons and a doublet of triplets due to couplings from two protons and one ^{13}C nucleus (see supplementary Fig. S1). However, the integrals revealed that the multiplet is



Scheme 2 Reagents and conditions: *a* $SOCl_2$, EtOH; *b* Boc_2O, Et_3N, CH_3OH; *c* $NaBH_4$, LiCl, MeOH; *d* HCl gas, CH_2Cl_2; *e* 48% HBr; *f* (1) cysteine, KOH, H_2O, (2) HCl

three times than the triplet. This means one triplet corresponding to the doublet of triplets is merged with the other triplet resulting in a multiplet. The reaction of **5** with cysteine produced 4-thia-[6-^{13}C]lysine hydrochloride (**6**) which was purified by cation exchange chromatography followed by repeated crystallization. Specific rotation of −3.9° was observed for the 1% solution of 4-thia-[6-^{13}C]lysine in water. In the literature there have been reports of two different values for unlabelled 4-thialysine. Cavallini et al. (1955) reported the value as +7.2°, while Lindley (1959) reported it as −4.4°. Therefore, we measured the commercially available unlabelled compound and found that the value is identical with that of 4-thia-[6-^{13}C]lysine.

We developed a scheme where all the steps have high yields. Interestingly, the intermediates, which are stable, can be easily purified avoiding cumbersome techniques like chromatography or continuous extraction. In this scheme, most of the reactions are performed in room temperature and milder reagents were used avoiding pyrophoric reagents like Pd/C or $LiAlH_4$. Moreover, previous report of preparation of 2-aminoethanol from glycine had the yield of 50%. We obtained the overall yield of 68%. This scheme essentially gives access to all possible isotopomers of 4-thialysine in which any of the stable isotopes such as ^{13}C, ^{15}N, ^{17}O, ^{18}O and ^{2}H or radioisotopes such as ^{14}C, ^{11}C, ^{3}H and ^{35}S can be incorporated in a site-directed way. Either starting with appropriate isotopomer of glycine or reacting with appropriate reagent such as NaB^2H_4, NaB^3H_4 or site-specific isotopomer of cysteine (Siebum et al. 2004b) would produce desired site-directed isotopomer of 4-thialysine. It is evident that the scheme also gives easy access to site-directed isotopomers of 2-aminoethanol and 2-bromoethylamine.

Biophysical study

4-Thia-[6-^{13}C]lysine has successfully mimicked the reaction of unlabeled 4-thialysine with lysine-5,6-aminomutase. The EPR spectra of the radical intermediates after 10 s of reaction of 4-thialysine, 4-thia-[6-^{13}C]lysine and 4-thia-[5-^{13}C]lysine with 5,6-LAM in the presence of [4′-^{2}H] pyridoxal-5′-phosphate are shown in Fig. 1. Absence of detectable line broadening in the case of 4-thia-[6-^{13}C]lysine with respect to that of 4-thialysine indicates that the spin is not present on carbon-6. On the contrary, the spectrum of 4-thia-[5-^{13}C]lysine showed observable line

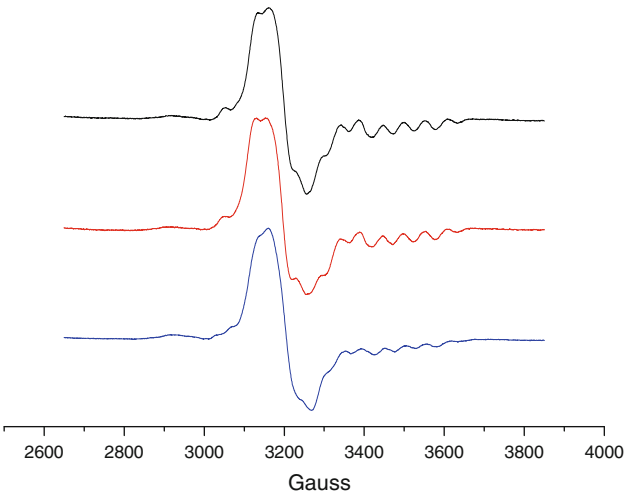


Fig. 1 X-band EPR spectra of radical intermediates in 5,6-LAM reaction with 4-thialysine (*top*), 4-thia-[6-^{13}C]lysine (*middle*) and 4-thia-[5-^{13}C]lysine (*bottom*) in the presence of [4′-^{2}H]pyridoxal-5′-phosphate. Experimental: microwave frequency, 9.536 GHz; power, 20 mW; modulation, 4 G at 100 kHz; $T = 80$ K

broadening which means the spin is on the carbon-5. Taken together EPR results suggest that radical intermediate is the thia-analog of substrate–PLP radical ($S^{\cdot}$).

Conclusion

In summary, we presented the synthesis of 4-thia-[6-^{13}C]lysine, a site-directed isotopomer of 4-thia analog of lysine. We also demonstrated that the same synthetic strategy would give access to all possible site-directed isotopomers of 2-aminoethanol, 2-bromoethylamine and 4-thialysine.

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