Synthesis of ^{13}C labeled β-cyano-ʟ-alanine

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Abstract: A synthetic methodology for preparation of β-cyano-ʟ-alanine was developed via ʟ -serine. Esterification of ʟ -serine with MeOH, followed by protection of NH_2 with CBz, gave methyl 2-(benzyloxycarbonylamino)-3-hydroxypropanoate. Mesylation of OH group, and then cyanation, ester hydrolysis and removal of the CBz group with catalytic hydrogenation, gave β-cyano-ʟ-alanine. Crucial step for the synthesis of the title compound was cyanation with $K^{13}CN$.

1. Introduction

Cyanide ion is surprisingly widely present in nature, and is assimilated through different biological processes, among which β-cyano-ʟ-alanine pathway is one of the best known processes. In this process, ethylene biosynthesis produces stoichiometrically cyanide ions which is then deactivated by enzymatically incorporation to L-cystein (**1**) through substitution.[1] In the further step, β-cyanoalanine (**2**) is hydrolysed by 3-cyanoalanine hydratase to give asparagine (**3**) (Scheme 1).[2]
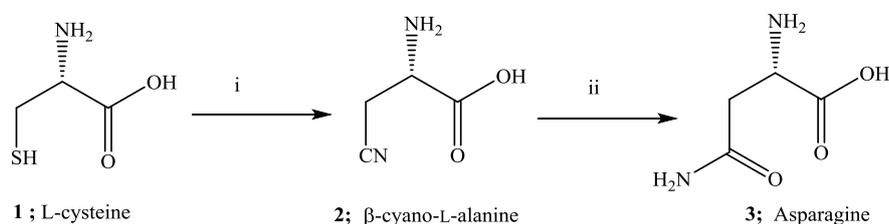
Scheme 1. Assimilation of HCN *via* β-cyano-ʟ-alanine pathway. (i) HCN, L-3-cyanoalanine synthase (ii) H_2O, 3-cyanoalanine hydratase

Chemical syntheses of β-cyano-ʟ-alanine are too restricted in the literature. In this context, carbobenzoxy-ʟ-asparagine[3], trityl-ʟ-asparagine[4], BOC-ʟ-asparagine[5], (o-nitrophenylsulfenyl)asparagine[6], and 3-amino-2-oxetanone salts[7] were used as the key compounds in the syntheses of β-cyanoalanine. β-Cyanoalanine labeled with ^{14}C or ^{15}N in the cyano group was prepared using immobilized cyanoalanine synthase (EC 4.4.1.9).[8] [14]C- and [15]C-labeled β-cyano-ʟ-alanine were prepared by cyanidation of labeled cysteine catalyzed by cyanoalanine synthase.[9] ^{11}C-labeled β-cyano-ʟ-alanine was prepared via β-cyano-ʟ-alanine synthase-catalyzed reaction of O-acetyl-ʟ-serine with labeled HCN.[10] β-Cyano-ʟ-alanine labeled with ^{14}C in C-4 was prepared based on a chemical synthesis.[11] β-[13C]cyano-ʟ-alanine was synthesized via acylase-catalyzed hydrolysis of $AcNHCH(CO_2H)CH_2^{13}CN$.[12]

Herein, we present an alternative synthetic methodology for preparation of β-[13C]cyano-ʟ-alanine as an isotopical standard in analytical determination of β-cyano-ʟ-alanine and γ-glutamyl-β-cyanoalanine[13].

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2. Experimental

2.1. Chemicals and Instrumentation

THF was purified and distilled over Na. All other solvents and reagents were used as received. Reactions were monitored by TLC, using TLC Merck silica gel 60 F254. Column chromatography was performed on silica gel 60 (0.063-0.2 mm). Column fractions were visualized using UV light (254 nm) and a solution of phosphomolybdic acid in ethanol (5 wt%). ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian 400 MHz instrument (400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR). All chemical shifts were reported in ppm and J values were given in Hz. Optical rotations were measured with a Bellingham + Stanley ADP220 spectropolarimeter (589 nm) at 25 °C. Melting points were determined on a Büchi 539 capillary melting apparatus and are uncorrected. All percent yields were calculated via isolated compounds.

2.2. Methyl 2-amino-3-hydroxypropanoate hydrochloride (5):

Under N_2 atmosphere and at 0 °C, acetyl chloride (9.80 mL, 10.8 g, 138 mmol) was added dropwise to a solution of L-serine (**4**) (5.00 g, 47.6 mmol) dissolved in MeOH (30 mL), and the mixture was stirred for about 10 min. The solution was then refluxed for 2 h. The mixture was cooled and the solvent was evaporated to afford **5** as a white solid (7.50 g, 100%). Compound **5** was used in the next step without further purification. m.p. 162 °C; Lit[16]. m.p. 161-162 °C.

2.3. Methyl 2-(benzyloxycarbonylamino)-3-hydroxypropanoate (6):

To a solution of methyl 2-amino-3-hydroxypropanoate (**5**) (3.00 g, 25.2 mmol) in THF (40 mL) was added a solution of saturated $NaHCO_3$ (40 mL) and benzyl chloroformate (4.30 mL, 5.15 g, 30.2 mmol) at 0°C, and then the mixture was warmed to 25°C and stirred for 3 h. The organic solvent was removed in vacuo and the residue was acidified to pH 2 by a solution of 3 M HCl, and the organic phases were extracted with EtOAc (3 × 70 mL). The combined organic phases were dried (Na_2SO_4) and evaporated in vacuo to afford yellow syrup. Chromatography of the crude product on a silica gel column (30 g) eluting with hexane/EtOAc (9:1) gave compound **6** as yellow oil (4.20 g, 66%). Rf = 0.27 (hexane-EtOAc, 4:1). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.37-7.26 (m, 5H, Ph), 5.97 (d, 1H, NH, $J_{H2,NH}$ = 7.8 Hz), 5.11 (bs, 2H, $PhCH_2O$), 4.41 (m, 1H, HC(2)), 3.95-3.83 (AB system, 2H, $H_2C(3)$, ^2J = 10.5 Hz), 3.72 (s, 3H, OCH_3), 3.16 (bs, 1H, OH). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.4 (C(1)), 156.6 (NH(CO)O), 136.3 (C(1')), 128.8 (C(3')/C(5')), 128.4 (C(2')/C(6')), 128.3 (C(4')), 67.4 ($PhCH_2O$), 63.2 (C(3)), 56.3 (C(2)), 52.9 (OCH_3). ^{1}H-NMR and ^{13}C-NMR of compound **6** were in agreement with the data given in the literature.[17]

2.4. Methyl 2-(benzyloxycarbonylamino)-3-(methylsulfonyloxy)propanoate (7):

CH_3SO_2Cl (1.70 mL, 2.5 g, 22.0 mmol) was added to a solution of methyl 2-(benzyloxycarbonylamino)-3-hydroxypropanoate (**6**) (3.73 g, 14.7 mmol) and NEt_3 (2.97 g, 28.4 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C, under N_2 atm. The reaction mixture was warmed to 25 °C and stirred for 3 h. The organic layer was extracted with CH_2Cl_2 (3 × 70 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to afford compound **7** as yellow oil (4.48 g, 92%). It was used in the next step without further purification.

2.5. Methyl 2-(benzyloxycarbonylamino)-3-cyanopropanoate (8):

Methyl 2-(benzyloxycarbonylamino)-3-(methylsulfonyloxy)propanoate (**7**) (3.60 g, 10.9 mmol) and NaCN (1.60 g, 32.6 mmol) were dissolved in 20 mL of dry N,N-dimethylformamide (DMF) under N_2 atm at 25 °C. After the solution was stirred for 14 h, DMF was removed in vacuo, and the organic layers were extracted with 3 × 70 mL of EtOAc. The combined organic phases were dried (Na_2SO_4) and evaporated under reduced pressure. Column chromatography of the crude product on a silica gel (30 g), eluting with hexane/EtOAc (8:2) gave compound **8** as yellow solid (2.17 g, 76%). Rf = 0.20 (hexane-EtOAc, 3:2). m.p. 92°C, Lit.[18] m.p. 91.5-92.5°C. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.39-7.26 (m, 5H, Ph), 5.81 (bs, 1H, NH), 5.16-5.09 (AB system, 2H, $PhCH_2O$, $J_{A,B}$ = 12.3 Hz), 4.58 (q, 1H, HC(2), J = 5.9 Hz),

3.83 (s, 3H, OCH$_3$), 3.01 (A part of AB system, dd, 1H, H$_a$C(3), $J_{3a,3b}$ = 16.8 Hz, J$_{2,3a}$ = 5.2 Hz), 2.94 (B part of AB system, dd, 1H, H$_b$C(3), $J_{3a,3b}$ = 16.8 Hz, J$_{2,3b}$ = 4.8 Hz). ^{13}C-NMR (100 MHz, CDCl$_3$): 169.3 (C(1)), 155.7 (NH(CO)O), 135.9 (C(1')), 128.8 (C(3')/C(5')), 128.7 (C(2')/C(6')), 128.4 (C(4')), 116.2 (CN), 67.8 (PhCH$_2$O), 53.7 (C(2)), 50.8 (OCH$_3$), 21.9 (C-3). ^{1}H-NMR and ^{13}C-NMR of compound **8** were in agreement with the data given in the literature.[19]

2.6. Methyl 2-(benzyloxycarbonylamino)-3-[^{13}C]cyanopropanoate (8):

^{1}H-NMR (400 MHz, CDCl$_3$): 7.38-7.32 (m, 5H, Ph), 5.72 (d, 1H, NH, J = 5.8 Hz), 5.16 (A part of AB system, d, 1H, PhCH$_2$O, J = 11.9 Hz), 5.12 (B part of AB system, d, 1H, PhCH$_2$O, J = 11.9 Hz), 4.59 (m, 1H, HC(2), 3.85 (s, 3H, OCH$_3$), 3.09-2.82 (m, 2H, H$_2$C(3)). ^{13}C-NMR: 116.2 ([13]CN), 21.9 (d, C(3), $J_{C\text{-}C}$ = 58.0 Hz).

2.7. 2-(Benzyloxycarbonylamino)-3-cyanopropanoic acid (9):

22.8 mL of 1 M KOH (22.8 mmol) was added to a solution of methyl 2-(benzyloxycarbonylamino)-3-cyanopropanoate (**8**) (2.00 g, 7.63 mmol) dissolved in 10 mL of EtOH. The solution was stirred for 3 h, after which the pH of the solution was adjusted to 2, using 4 M HCl solution, the organic layer was extracted with EtOAc (3 × 70 mL), dried over Na$_2$SO$_4$, and the solvent was evaporated under reduced pressure. Column chromatography of the crude product on a silica gel (20 g), eluting with hexane-EtOAc (8:2), gave compound **9** as yellow solid (1.37 g, 72%). m.p. 132.5°C. Lit.[18] m.p. 132-133.5°C. ^{1}H-NMR (400 MHz, CDCl$_3$): 7.40-7.30 (m, 5H, Ph), 5.78 (d, 1H, NH, J = 7.3 Hz), 5.14 (bs, 2H, PhCH$_2$O), 4.62 (q, 1H, HC(2), J = 7.0 Hz), 3.07 (A part of AB system, dd, 1H, H$_a$C(3), $J_{3a,3b}$ = 16.0 Hz, $J_{2,3a}$ = 4.7 Hz), 2.99 (B part of AB system, dd, 1H, H$_b$C(3), $J_{3a,3b}$ = 16.0 Hz, $J_{2,3b}$ = 5.1 Hz). ^{13}C-NMR (100 MHz, CDCl$_3$): 171.6 (C(1)), 155.9 (NH(CO)O), 135.7 (C(1')), 128.9 (C(3')/C(5')), 128.7 (C(2')/C(6')), 128.4 (C(4')), 116.1 (CN), 68.0 (PhCH$_2$O), 50.6 (C(2)), 21.8 (C(3)). ^{1}H-NMR of compound **8** was in agreement with the literature data.[19]

2.8. 2-(Benzyloxycarbonylamino)-3-[^{13}C]cyanopropanoic acid (9):

^{1}H-NMR (400 MHz, DMSO-d$_6$): 13.20 (bs, 1H, COOH), 7.98 (d, 1H, NH, J = 8.4 Hz), 7.39-7.30 (m, 5H, Ph), 5.09-5.00 (m, 2H, PhCH$_2$O), 4.38-4.31 (m, 1H, HC(2)), 2.99-2.79 (m, 2H, H$_2$C(3)). ^{13}C-NMR (100 MHz, DMSO-d$_6$): 118.8 ([13]CN), 20.6 (d, C(3), $J_{C\text{-}C}$ = 58.0 Hz).

2.9. 2-Amino-3-cyanopropanoic acid (β-cyano-L-alanine) (2):

To a solution of 2-(benzyloxycarbonylamino)-3-cyanopropanoic acid (**9**) (1.00 g, 4.00 mmol) in 30 mL of MeOH was added 10% Pd-C (0.10 g). After removal of O$_2$ by flushing with N$_2$, the reaction mixture was hydrogenated under H$_2$ atmosphere (1 atm) for 14 h. The catalyst was removed by filtration and the mixture was washed with MeOH, the solvent was evaporated and the residue was purified by soluting in H$_2$O and participation with dioxane (three times) to give 2-amino-3-cyanopropanoic acid (**2**) and 2,4-diamino-butanoic acid (**10**)[15] in a ratio of 95:5 (0.17 g, 37%), m.p. 212°C. Lit.[7] m.p. 217-218°C. dec. ^{1}H-NMR (400 MHz, D$_2$O): 4.64 (bs, 2H, NH$_2$ and COOH), 3.92 (t, 1H, HC(2), J = 5.8 Hz), 3.00 (d, 2H, H$_2$C(3), J = 5.5 Hz). ^{13}C-NMR (100 MHz, D$_2$O): 171.8 (C(1)), 117.4 (CN), 50.8 (C(2)), 20.0 (C(3)). ^{1}H-NMR of compound **2** was in agreement with the data given in the literature.[7] ^{13}C-NMR of compound **2** was in agreement with the data given in the literature.[20]

2.10. 2-Amino-3-[^{13}C]cyanopropanoic acid (2):

^{1}H-NMR (400 MHz, D$_2$O): 4.80-4.55 (bs, NH$_2$, COOH), 3.87 (q, 1H, HC(2), J = 7.4 Hz), 2.95 (dd, 1H, H$_2$C(3), J = 9.9 Hz; 6.2 Hz). ^{13}C-NMR (100 MHz, D$_2$O): 116.9 ([13]CN), 20.6 (d, C(3), $J_{C\text{-}C}$ = 58.0 Hz). HRMS calc. for C$_3$ [13]C H$_5$ O$_2$ N$_2$, 114.0379; found, 114.0374.

3. Results and Discussion
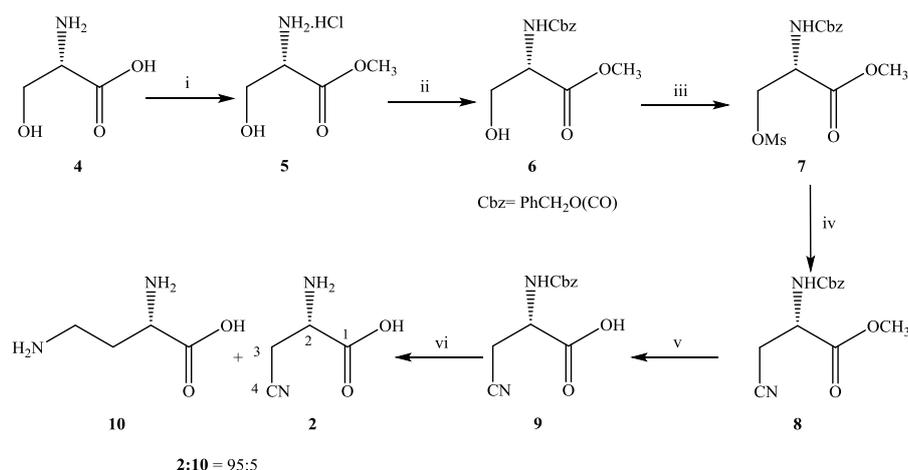
Scheme 2. β-Cyano-ʟ-alanine *via* ʟ-serine. (i) AcCl, MeOH, 0°C → reflux, 2 h, 100%; (ii) PhCH₂O(CO)Cl, NaHCO₃, THF, 0°C → rt, 3 h, 66%; (iii) CH₃SO₂Cl, NEt₃, dry CH₂Cl₂, 0°C → rt, 3 h, 92%; (iv) NaCN (or K¹³CN), dry DMF, rt, 14 h, 76%; (v) KOH, EtOH, rt, 3 h, then 4 M HCl 72%; (vi) H₂/Pd-C, MeOH, rt, 14 h, 37%.

Chemical synthesis of β-cyano-ʟ-alanine (**2**) is outlined in Scheme 2. In the synthesis, ʟ-serine was used as a starting material. Initially, ʟ-serine was converted to ʟ-serine methyl ester hydrochloride (**5**) by treatment with MeOH/AcCl. Amine functionals are often protected by carboxybenzyl (Cbz) group as carbamates and deprotected by Pd-C-catalyzed hydrogenation or HBr treatment.[14] By applying this methodology, ester **5** was treated with carboxybenzyl chloride (CbzCl) to give NH-Cbz ʟ-serine methyl ester (**6**). Compound **6** was converted to mesylate ester **7** by reacting with MeSO₂Cl. Mesylate **7** was subjected to substitution with NaCN to give cyanide **8**. Thus, all functionalities were constructed in the molecule. At this stage, hydrolysis of the ester and removal of the Cbz group are needed to reach to target molecule **2**. For this purpose, first, ester **8** was converted to acid **9** by hydrolysis with ethanolic KOH, followed by acidification with HCl, and then N-Cbz compound **9** was hydrogenated with Pd-C/H₂ to remove the Cbz group. In the hydrogenation of **9**, the target molecule, β-cyano-ʟ-alanine (**2**), was formed as the main product with a small amount of side product. ¹³C-NMR of the side product was in good agreement with the data given for 2,4-diaminobutyric acid (**10**).[15] Due to compound **10**, being the reduced derivative of **2**, we suppose that compound **10** is formed by the reduction of the CN group. NMR analysis showed the ratio of the products as **2:10** = 95:5. When K¹³CN was used in the substitution of mesylate **7** in the same conditions, [¹³CN]-labeled **8** was formed. ¹³C-labeled derivatives of **9** and **2** were also obtained by the same reaction sequence. C-3 carbons of compounds **8**, **9**, and **2** resonated as doublets with J_{C-C} = 58 Hz, which confirms the incorporation of ¹³CN into the molecules.

In previous studies, ¹³C labeled β-cyano-ʟ-alanine (**2**) was synthesized through enzymatic transformation.[12] On the other hand, this study exhibits a straightforward chemical synthesis of β-cyano-ʟ-alanine (**2**) *via* ʟ-serine. Therefore, the present synthetic methodology can be readily applicable to new synthetic amino acid derivatives based on ʟ-serine.

Acknowledgements

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/OC

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