SHORT COMMUNICATION

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Org. Commun. 13:4 (2020) 202-206

organic communications

Synthesis of ɣ-glutamyl β-cyanoalanine precursor

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(Received May 19, 2020; December 12, 2020; Accepted December 24, 2020)

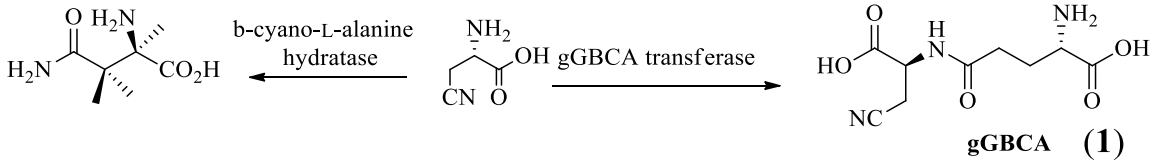
Abstract: The synthesis of precursors of neurotoxic ɣ-glutamyl β-cyanoalanine was developed starting from L-Serine via through the preparation of β-cyanoalanine and glutamyl units. Coupling of these two intermediates gave methyl ester of ɣ-glutamyl β-cyanoalanine precursor.

Keywords: Amino acid; β-cyanoalanin (BCA); precursors of gGBCA; ɣ-glutamyl β-cyanoalanine (gGBCA).

1. Introduction

Vicia sativa contains remarkable amount of β-cyanoalanine (BCA, ~0.97%) and γ-glutamyl β-cyanoalanine (gGBCA, ~2.6%),[1] secondary metabolites are important chemical compounds produced by plant kingdom[2-4], however, neurotoxicities of which on animals and humans due to cyanide moiety of amino acids were reported.[5-9] In plant metabolism, BCA is produced stoichiometrically via ethylene biosynthesis.[1] In a further process, BCA is converted to asparagine by β-cyanoalanine hydratase enzyme in the some species, *Vicia sativa*, which is then converted to gGBCA by glutamyl transferase enzyme (Scheme 1).

While the synthesis of ^{13}C labelled β-cyanoalanine in five steps starting from L-serine was disclosed by Ghasemi and Secen for determination of the neurotoxic activity,[10,11] its LC-MS-MS characterization method was reported by our group.[11] On the other hand, to our best knowledge, total synthesis of gGBCA has not been reported yet. In this study, a methodology aimed for the total synthesis of γ-glutamyl β-cyanoalanine (gGBCA) was reported. Unfortunately, the last step involving hydrolysis of methyl ester of gGBCA to obtain the target compound, ɣ-glutamyl β-cyanoalanine (gGBCA), could not be achieved, efficiently.



Scheme 1. Metabolic synthesis of asparagine and γ-glutamyl β-cyanoalanine in higher plants

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The article was published by ACG Publications
http://www.acgpubs.org/journal/organic-communications © October December 2020 EISSN:1307-6175
DOI: http://doi.org/10.25135/acg.oc.89.20.05.1660

2. Experimental

Chemical Materials and Apparatus: Chemicals and solvents were obtained from commercially available sources and used without further purification. Thin layer chromatography (TLC) was performed using Merck Silica gel plates (Merck 60, 0.25 mm thickness) with F254 indicator. Column chromatography was applied on silica gel (0.063-0.200 mm). Mass analyses were recorded on a Thermo Scientific Q Exactive instrument. ^{1}H and ^{13}C NMR spectra of all the compounds were recorded in $CDCl_3$ and D_2O solutions on a Varian VNMRs 600 MHz sectrometer.

Compound **3**-**5**, **9** and **10** were synthesized according to the reported protocols.[10-14]

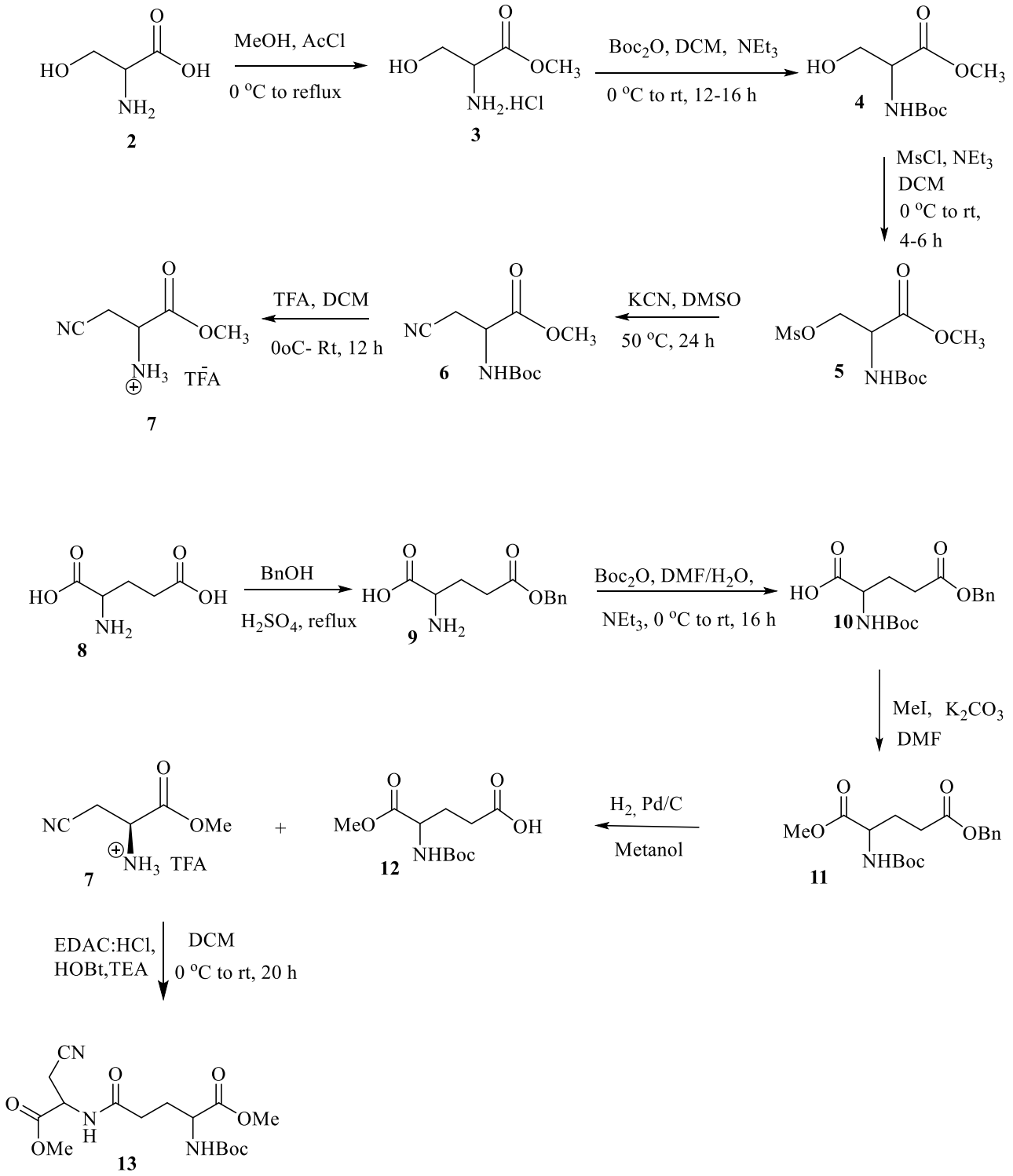
*Compound **3***: **2** (5 g, 47.6 mmol) was dissolved in MeOH (30 mL) under argon atmosphere at 0 °C. To this solution, acetyl chloride (9.80 mL, 10.8 g, 138 mmol) was added dropwise at 0 °C. The mixture was stirred for 15 min, after which it was allow to reach room temperature and the reaction mixture was refluxed for 2 h. Then, the solvent was removed under reduced pressure to give a white solid (98%, 7.2 g, mp:161-162 °C).^{1}H NMR (600 MHz, D_2O) δ ppm; 4.13-4.15 (m, 1H, CH), 3.92-4.02 (m, 2H, CH_2), 3.83 (s, 3H, OCH_3). ^{13}C-NMR (125 MHz, D_2O) δ 170.65 (C), 61.91 (CH_2), 57.34 (CH), 55.04 (CH_3).

*Compound **4***: **3** (2 g, 12.8 mmol) was dissolved in dry CH_2Cl_2 (30 mL) at 0 °C. To this solution di-*tert*-butyl dicarbonate (3.4g, 15.4 mmol) and NEt_3 (2.6 g, 25.7 mmol) were added at the same temperature. After the mixture was stirred overnight at room temperature, it was extracted with DCM (2x40 mL) and water (50 mL). The organic layer was dried over sodium sulfate, filtered and the mixture was concentrated under reduced pressure. The crude product was purified over columun chromatıoraphy packed with silica gel using EtOAc:Hexane (1:3) mixture as an eluent to give the pure compound **4** (78%, oil, 2.2 g, Rf: 0.25). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm; 5.55 (bs, 1H, NH), 4.32 (bs, 1H, CH), 3.81-3.90 (bs, 2H, CH_2), 3.73 (s, 3H, OCH_3) 3.01 (bs, 1H, OH), 3.72 (s, 9H, C-CH_3).

*Compound **5***: **4** (1 g, 4.56 mmol) was dissolved in dry CH_2Cl_2 (20 mL) at 0 °C. To this solution NEt_3 (0.92 g, 9.12 mmol) and $MeSO_2Cl$ (0.62 g, 5.47 mmol) were added at the same temperature. After the reaction mixture was stirred at room temperature for 4 h, it was extracted with DCM (3x40 mL) and water (50 mL). The organic layer was dried over sodium sulfate, filtered and the solvent was removed under reduced pressure to give a white solid (1.0 g, 73.8%, R*f*: 0.32, EtOAc:Hexane 1:3). It was used in next step without further purification.

*Compound **6***: **5** (0.22 g, 0.75 mmol) was dissolved in *N*,*N*-dimethylformamide (5 mL). To this solution, potassium cyanide (0.1g, 1.49 mmol) was added at room temperature and the resulting mixture was stirred for 24 h at 40-50 °C. The reaction mixture was cooled to room temperature and diluted with water. It was then extracted with ethyl acetate (3x20 mL) and washed with water (50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified over columun chromatography packed with silica gel using EtOAc:Hexane (1:3) mixture as an eluent to give pure compound **6** as a white solid (81.4%, 0.14 g, R*f*: 0.50, EtOAc:Hexane 1:3). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm; 5.42 (bs, 1H, NH), 4.47 (bs, 1H, CH), 3.78 (s, 3H, OCH_3), 2.83-2.97 (m, 2H, CH_2), 1.39 (s, 9H, C-CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.41 ($CH_3O(\underline{C}O)$), 154.81 ($NH(\underline{C}O)O$), 116.19 (CN),81.06 ($\underline{C}O(CH_3)_3$), 53.39 (CH), 50.28 (OCH_3), 28.22 ($OC(\underline{C}H_3)_3$), 21.90 (CH_2).

*Compound **7***: Trifluoroacetic acid (0.5 mL) was added to a solution of **6** (0.1 g, 1.32 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C, and the mixture was stirred at room temperature for 15 h. It was then concentrated under reduced pressure, washed with diethyl ether and the solvent was evaporated under reduced pressure to give **7** (0.05 g, 52%) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ ppm; 4.52 (t, 1H, CH), 3.83 (s, 3H, OCH_3), 3.23 d, 2H, CH_2).



Scheme 2. Synthesis of ɣ-glutamyl β-cyanoalanine precursor

Compound ***9***: Benzyl alcohol (50 mL) was added to a solution of concentrated H_2SO_4 (5 mL) dissolved in diethyl ether (50 mL). Then, the mixture was concentrated under reduced pressure, to which L-glutamic acid (**8**) (7.35 g, 50 mmol) was added portionwise over a period of 15 min. The mixture was stirred at room temperature for 20 h, diluted with ethanol (100 mL), and, then, pyridine (25 mL) was added under vigorous stirring. The mixture was cooled to 0 °C and left stirring overnight at this temperature. The resulting precipitate was filtered and washed with ether to afford **9** (60%, 7.2 g) as a white solid, ^{1}H NMR (600 MHz,

D_2O) δ ppm; 7.30-7.26 (m, 5H, Ar), 5.03 (s, 2H, O-$\underline{CH_2}$-Ph), 3.62 (t, 1H, CH), 2.45 (dd, 2H, CH_2), 2.04-1.98 (m, 2H, CH_2).

*Compound **10***: To a solution of compound **9** (4.5 g, 18.9 mmol) in 100 mL of DMF/ H_2O (1:1) was added di-*tert*-butyl dicarbonate (4.16 g, 19.05 mmol) and NEt_3 (1.96 g, 19.4 mmol) at 0 °C. The resulting mixture was stirred at room temperature overnight and, then, the solvent was evaporated under reduced pressure to afford an oily residue, which was suspended in ethyl acetate and acidified to pH 2 with aqueous HCl solution. The mixture was extracted with ethyl acetate (2 x 50 mL), washed with H_2O (50 mL), dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to obtain compound **10** (63%, 4.2 g) as an oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm; 5.55 (bs, 1H, NH), 4.32 (bs, 1H, CH), 3.81-3.90 (bs, 2H, CH_2), 3.73 (s, 3H, OCH3), δ= 3.01 (bs, 1H, OH), 3.72 (s, 9H, C-CH3).$C_9H_{13}O$.

*Compound **11***: To a solution of **10** (1.6 g, 4.75 mmol) in DMF (50 mL) at 0 °C was added potassium carbonate (0.70 g, 5.02 mmol). After the suspension was stirred for 10 min in an ice-water bath, methyl iodide (0.3 mL, 4.75 mmol) was added and stirring was continued for 30 min. The mixture was allowed to reach room temperature, then, the stirring was continued overnight. The mixture was filtered and the filtrate was portioned between ethyl acetate (30 mL) and water (30 mL). The organic layer was washed with brine (2 x20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give compound **11** (60%, 1.0 g) as an oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm; 7.27-7.33 (m, 5H, Ar), 5.03(s, 2H, O-$\underline{CH_2}$-Ph), 4.27 (m, 1H, CH),3.65 (s, 3H, OCH_3), 2.26-2.41 (m, 2H, CH_2), 2.15,1.91 (m, 2H, CH_2), 1.36 (s, 9H, C-CH_3).

*Compound **12:*** A mixture of **11** (1g, 2.85 mmol) and palladium-carbon (100 mg, 10%) in 15 mL of methanol was stirred overnight under hydrogen atmosphere. The catalyst was filtred and the solvent was evaporated under reduced pressure to give **c**ompound **12** as an oil in 54% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm; 4.67-4.72 (b, H, NH), 5.03(s, 2H, O-$\underline{CH_2}$-Ph), 4.24-4.27 (m, 1H, CH),3.66 (s, 3H, OCH_3), 2.29-2.43 (m, 2H, CH_2), 2.12,1.85 (m, 2H, CH_2), 1.35 (s, 9H, C-CH_3).

*Compound **13***: To a solution of **12** (0.22 g, 0.83 mmol) in CH_2Cl_2 (20 mL) was added EDAC.HCl (0.190 g, 0.99 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. Then, 2-hydroxybenzotriazole hydrate (HOBt, 0.112 g, 0.83 mmol) was added and the mixture was stirred at 0 °C for 1 h, after which **7** (0.2 g, 0.83 mmol) and triethylamine (0.12 g, 0.99 mmol) were added, and the resulting solution was stirred at room temperature for 20-24 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution, water, aqueous 10% citric acid and water. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solution was concentrated under reduced pressure and the residue was purified by column chromatography packed with silica gel to give the pure compound **13** as a white solid (51%, 0.16 g, R*f*: 0.26, EtOAc:Hexane 2:3). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm; 4.73-4.65 (m, H, CH), 4.31-4.26 (b,1H, NH), 4.15-4.06 (m, H, CH), 3.77 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.02-2.86 (m, 2H, CH_2), 2.40-2.32 (m, 2H, CH_2), 2.20,1.89 (m, 2H, CH_2), 1.38 (s, 9H, C-CH_3). ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm; (171.68, 171.35, 168.32, 155.03) (-CO), 115.50 (-CN), 79.30 (tert-C-), (59.41) (-CH-), 52.18, 51.20 (-OMe), (48.18) (-CH-), 30.81 (-CH_2-), 27.84(-$C(CCH_3)_3$), (27.26, 20.06) (-CH_2-).

3. Present Study

Herein, synthesis of ɣ-glutamyl β-cyanoalanine precursor **13** was reported for the first time. The synthesis was achieved starting from serine (**2**). A synthetic protocol of **3**-**7** was reported previously in the literature.[7,9-12] After the synthesis of β-cyanoalanine derivative (**7**), *N-tert*-butoxycarbonyl-ɣ-benzyl-L-glutamic acid (**10**) was synthesized and carboxylic acid group of **10** was converted to methyl ester using CH_3I, under basic condition. Approximately one gram of the product was obtained (**11**, 60%). Compounds **12** and **13** were synthesized as described in section 3 and Scheme 2. The final step was the most problematic in the synthesis of ɣ-glutamyl β-cyanoalanine. β-cyanoalanine and its derivatives were easily converted to L-aspartic acid and its derivatives by acidic hydrolysis, and then the same problem as given in the literature was encountered.[4] Unfortunately, all attempts to remove the protecting groups of **13** to obtain the final product gGBCA did not proceed efficiently under basic hydrolysis. It could be attributed to the hydrolysis of nitrile group.

In conclusion, we have developed a synthetic route for the synthesis of gGBCA precursor which is ready to be used after the removal of deprotection groups. Further work on the hyrolysis of protection groups to access gGBCA will be reported in due course.

Acknowledgements

The authors would like to thank the T.C. Ministry of Agriculture and Forestry, Research and Development Support Program for financial support (grant no: TAGEM -18/AR-GE/15).

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/organic-communications

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