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MULTIGRAM-SCALE AND COLUMN CHROMATOGRAPHY-FREE SYNTHESIS OF L-AZETIDINE-2-CARBOXYLIC ACID FOR THE SYNTHESIS OF NICOTIANAMINE AND ITS DERIVATIVES

Tomohiro Takaishi,[1] Kyosuke Wakisaka,[1] Christopher J. Vavricka,[2] Hiromasa Kiyota,[1] and Minoru Izumi[1]*

[1] Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan. [2] Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan. E-mail: mizumi@okayama-u.ac.jp.

Abstract – Multigram-scale synthesis of L-azetidine-2-carboxylic acid from L-aspartic acid was achieved in 13 conventional synthetic steps, without the need for purification by silica-gel column chromatography and expensive reagents. Nicotianamine and its fluorescence-labeled derivatives could be obtained from this synthetic strategy.

Nicotianamine (**1**),[1] which is biosynthesized from three molecules of (*S*)-adenosyl-L-methionine via nicotianamine synthase, is a metal-chelating molecule ubiquitous in higher plants.[2] L-Azetidine-2-carboxylic acid (L-Aze **2**) is a key chemical precursor to nicotianamine, deoxymugineic acid, as well as nicotinic receptor tracers.[3] There are many reports on the organic synthesis of L-Aze and its derivatives; for example, Futamura *et al.* reported an efficient route to L-Aze in five steps (total yield: 48%) via malonic ester intermediates.[4] Recently, Bouazaoui *et al*. has reported the synthesis of L-Aze using the (2-trimethylsilyl)ethanesulfonyl (SES) protecting group as a leaving group on the hydroxy function and as an activator for the amine function.[5] It is necessary to develop a large-scale synthesis to enable medicine and agrochemical studies; however, it is desirable to obtain L-Aze and its derivatives without use of hydrogenolysis by Pd-C, selective enzymatic hydrolysis, and/or expensive reagents. In this study, we described an efficient and cheap route to L-Aze via L-aspartic acid intermediates and its application to the synthesis of nicotianamine.

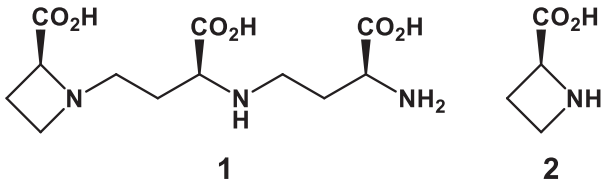
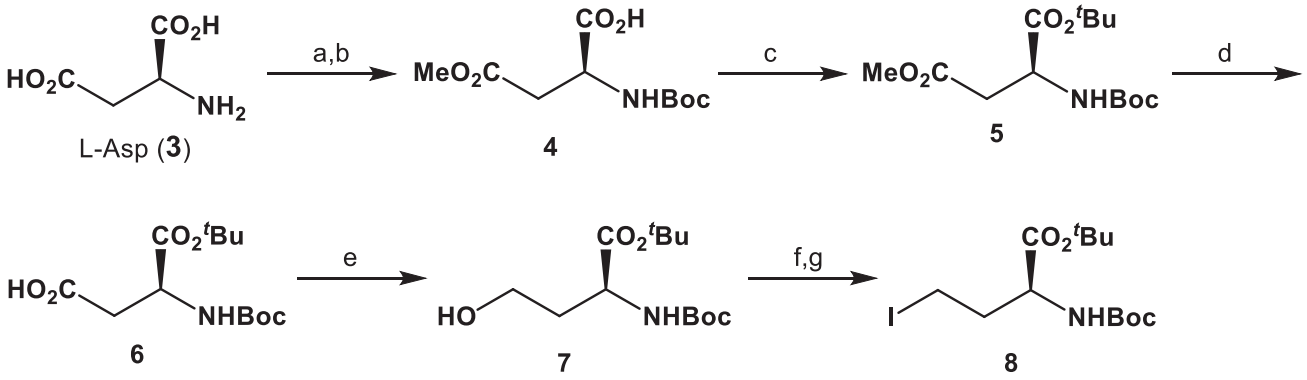


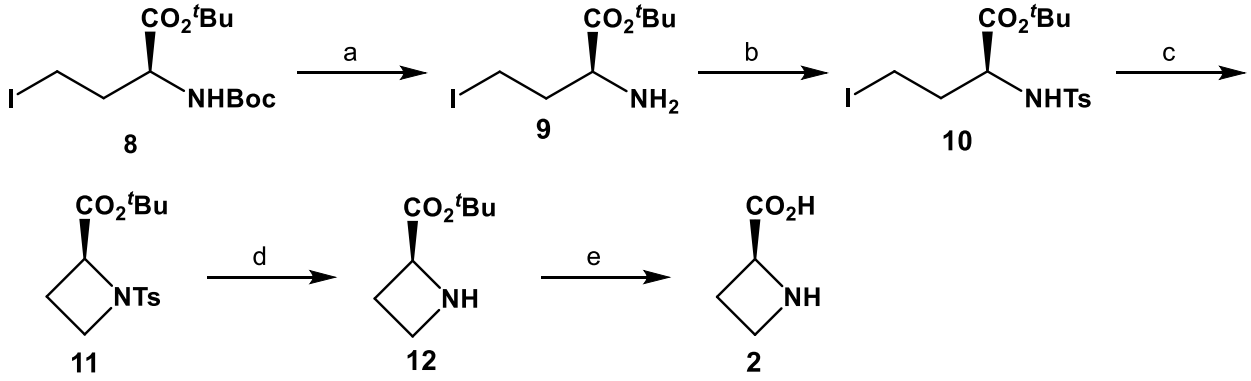
Figure 1. Nicotianamine (**1**) and L-azetidine-2-carboxylic acid (**2**)

Commercially available 1-*tert*-butyl L-aspartate or L-homoserine have recently been used as starting material for the synthesis of L-Aze and its derivatives. Bouazaoui *et al.* has reported good results using 1-*tert*-butyl L-aspartate and SES as key reagents,[5] however, the newly reported synthesis of this study started from 1 mole of L-aspartic acid (**3**) to reduce cost (Scheme 1). Methyl *N*-Boc-L-aspartate (**4**) was obtained in 99% yield over two steps after selective esterification using chlorotrimethylsilane (TMSCl) and protection of the amino group using di-*tert*-butyl dicarbonate ($(Boc)_2O$).[6] *tert*-Butyl esterification of α-carboxyl group followed by saponification of the methoxycarbonyl group located at β-position, produced the desired compound **6** in 92% yield (2 steps from **4**). Methyl *N*-Boc-L-aspartate and its derivatives could be easily separated by pH-controlled extraction from the reaction mixture without the need for silica-gel column chromatography. A chromatography-free process is idea for large-scale synthesis. Reduction of the carboxy moiety with $NaBH_4$ via the formation of a mixed acid anhydride derivative, followed by reaction with ethyl chloroformate in the presence of triethylamine, gave *tert*-butyl *N*-Boc-L-homoserine (**7**) in 84% yield. Among the many kinds of C-N bond formation for intramolecular cyclization (Mitsunobu reaction, reductive amination, etc.), direct *N*-alkylation was selected. Two steps, reaction of hydroxy group with *p*-TsCl followed by iodination, were necessary to afford the desired intermediate (**8**) in 91% yield (Scheme 1, overall yield of 77%, 7 steps from **3**).



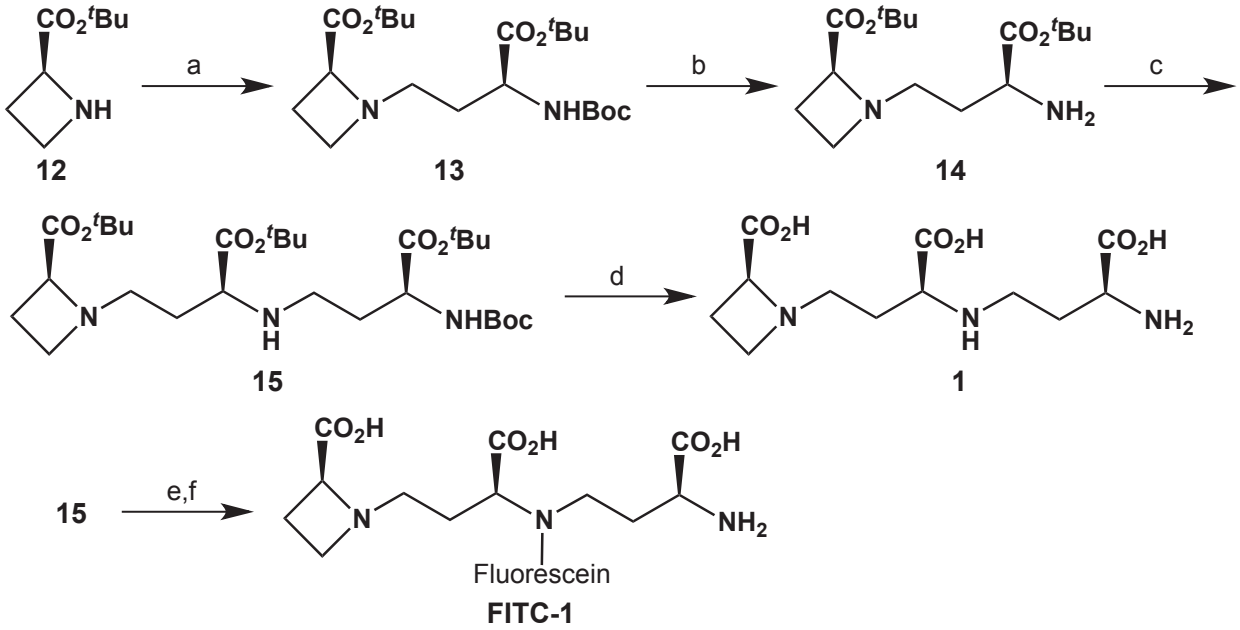
Scheme 1. Reagents and conditions: (a) TMSCl, MeOH, rt; (b) $(Boc)_2O$, dioxane, $NaHCO_3$ aq., 0 °C then rt, 99% (2 steps); (c) DCC, DMAP, *t*-BuOH, DCE, rt, 92%; (d) 1 M NaOH aq., acetone, rt, quant; (e) ethyl chloroformate, TEA, THF, 0 °C then $NaBH_4$, 0 °C then rt, 84%; (f) TsCl, TEA, DMAP; (g) NaI, acetone, rt, 91% (2 steps).

In the case of *N*-Boc derivatives, intramolecular cyclization did not occur due to low nucleophilicity of the *N*-Boc-amino group. Therefore, the amino protective group was changed Ts instead of SES or Ns groups[5,7] as TsCl was much cheaper than SESCl or NsCl. *N*-Ts intermediate (**10**) was obtained quantitatively after deprotection by TMSCl followed by TsCl amino re-protection. Cyclization of **10** was performed using Cs_2CO_3 in MeCN to give *N*-Ts-L-Aze (**11**) in 94% yield. Ts deprotection via Mg in MeOH[7] and *tert*-butyl deprotection via TFA afforded multigram quantities of L-Aze (**2**), obtained over 13 steps with an overall yield of 49% from L-aspartic acid **3** (Scheme 2).



Scheme 2. Reagents and conditions: (a) TMSCl, THF, MeOH, rt, quant; (b) TsCl, TEA, DMAP, DCE, 0 °C then rt, 94%; (c) Cs_2CO_3, MeCN, rt, quant; (d) Mg, MeOH, rt, 68%; (e) TFA, H_2O, DCE, quant.

Next, we compared the synthesis of nicotianamine (**1**) according to Bouazaoui's method[8] (Scheme 3). In the first alkylation step, *tert*-butyl L-azetidine-2-carboxylate (**12**) was successfully *N*-alkylated with the iodide **8**, together with DIPEA[9] in MeCN, to provide **13** in 60% yield. After deprotection of the *N*-Boc group quantitatively (**14**), the targeted compound (**15**) was obtained in 42% yield by the second *N*-alkylation step. Removal of the Boc group and *tert*-butyl groups proceeded without difficulty by TFA treatment to afford nicotianamine (**1**) quantitatively after lyophilization as a white powder. In order to identify the localization of nicotianamine in plant, we synthesized fluorescence-labeled nicotianamine (**FITC-1**) as chemical probe. After fluorescence labeling of the secondary amino group by fluorescein isothiocyanate, deprotection of *tert*-butyl groups proceeded by TFA treatment to afford FITC-labeled nicotianamine (**FITC-1**).



Scheme 3. Reagents and conditions: (a) **8**, DIPEA, MeCN, rt, 60%; (b) TMSCl, THF, MeOH, rt, quant; (c) **8**, Cs_2CO_3, MeCN, rt, 42%; (d) TFA, H_2O, DCE, rt, quant; (e) fluorescein 5-isothiocyanate, THF, rt; (f) TFA, H_2O, DCE, rt, 98% (2 steps).

In summary, multigram-scale synthesis of L-azetidine-2-carboxylic acid (**2**) from L-aspartic acid succeeded in 13 steps without the need for purification by column chromatography. Nicotianamine (**1**) and its fluorescence-labeled derivative (**FITC-1**) could be obtained via this method.

EXPERIMENTAL

Compounds were analyzed using the Varian 400 MHz systems at the Okayama University Collaboration Center. ^{1}H NMR (operating at 400 MHz) and ^{13}C NMR (operating at 100 MHz) spectra were measured in $CDCl_3$, D_2O or DMSO. NMR chemical shifts (δ) are provided in parts per million (ppm), and coupling constants (*J*) are listed in Hz. Residual peaks of chloroform (7.26 ppm), H_2O (4.63 ppm) or DMSO (2.49 ppm) were used as ^{1}H-NMR references. The melting points were obtained on Yamato melting point MP-21 apparatus. Optical rotations values were measured with a JASCO P-2200 apparatus (20 ^{o}C, sodium discharge lamp). TLC was carried out on Merck Kieselgel 60 PF_{254} (0.25 mm thickness). Column chromatography was performed using Merck Kieselgel 60 (0.063–0.200 mm). All chemicals used in this study were commercially available, and all of the organic solvents used in this study were dried over appropriate drying agents and distilled prior to use.

Methyl *N*-Boc-L-aspartate (4). Compound **4** was prepared by the appropriate reported procedure.[6] To a mixture of L-aspartic acid (13.3 g, 0.10 mol) in MeOH (100 mL) was added TMSCl (25 mL, 0.20 mol) at room temperature. The resulting mixture was stirred at room temperature for 6 h, and then concentrated

under reduced pressure. To a mixture of the residue in 1,4-dioxane (67 mL) and sat. $NaHCO_3$ aq. (33 mL) was added a solution of di-*tert*-butyl dicarbonate (24.0 g, 0.11 mmol) in 1,4-dioxane (10 mL) at 0 ºC. The reaction mixture was stirred at 0 ºC for 1 h and then stirred at room temperature for 24 h. The 1,4-dioxane was removed on a rotary evaporator, the residue was poured onto ice-water (100 mL) and the cold solution was washed with $CHCl_3$ (100 mL × 3) to separate nonacidic compounds. The aqueous solution was acidified (pH 2.5) with a solution of 1 M HCl aq., and extracted with $CHCl_3$ (100 mL × 5). The combined $CHCl_3$ extracts were washed with water and dried. The solvent was removed on a rotary evaporator to give the *N*-Boc-monomethyl ester as a colorless liquid (**4**, 24.8 g, 0.11 mmol, quant); ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s, *t*-Bu), 2.81 (1H, dd, *J* = 4.8, 17.2 Hz, Hβ-a), 3.01 (1H, dd, *J* = 4.8, 17.2 Hz, Hβ-b), 3.64 (3H, s, CO_2Me), 4.20 (1H, t, *J* = 5.5 Hz, Hα); ^{13}C NMR ($CDCl_3$) δ 28.4, 36.6, 51.9, 54.3, 79.5, 156.0, 169.3, 173.2.

***tert*-Butyl *N*-Boc-L-aspartate 4-methyl ester (5).** To a solution of compound **4** (88.2 g, 0.36 mol) in DCE (400 mL) were added DCC (81.0 g, 0.40 mol), DMAP (4.36 g, 0.036 mol) and *tert*-butyl alcohol (73.0 mL, 0.72 mol) at 0 ºC. The resulting mixture was stirred at room temperature for 24 h, and then concentrated under reduced pressure. The residue was dissolved into AcOEt (500 mL) and the cold solution was washed with a solution of 1 M HCl aq. (250 mL), and brine (250 mL), and dried. The solvent was removed on a rotary evaporator to give the *N*-Boc-diester as a colorless solid (99.9 g, 92%); mp 68-70 ºC; ^{1}H NMR ($CDCl_3$) δ 1.45 (12H, s, *t*-Bu), 1.68 (6H, s, *t*-Bu), 2.81 (1H, dd, *J* = 4.8, 17.2 Hz, Hβ-a), 3.01 (1 H, dd, *J* = 4.8, 17.2 Hz, Hβ-b), 3.64 (3H, s, CO_2Me), 4.20 (1H, t, *J* = 5.5 Hz, Hα); ^{13}C NMR ($CDCl_3$) δ 28.4, 36.1, 51.7, 54.2, 79.4, 155.7, 169.1, 169.7.

***tert*-Butyl (*S*)-2-(*tert*-butoxycarbonylamino)-4-hydroxybutanoate (6).** To a solution of compound **5** (99.9 g, 0.33 mol) in acetone (400 mL) and H_2O (50 mL) was added 1 M NaOH aq. (175 mL) at room temperature. The reaction mixture was stirred at room temperature for 4.5 h, and then concentrated under reduced pressure. The residue was solved in $CHCl_3$ (400 mL), and the solution was washed with 1 M HCl aq. (100 mL × 3) and dried. The solvent was removed on a rotary evaporator, and the residue was crystallized from hexane to give the *tert*-butyl *N*-Boc-L-aspartate **6** as a colorless solid (84.4 g, quant); mp 98-102 ºC; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 12 H, *t*-Bu), 1.68 (6H, s, *t*-Bu), 2.81 (1H, dd, *J* = 4.4, 17.0 Hz, Hβ-a), 3.01 (1H, dd, *J* = 4.4, 17.0 Hz, Hβ-b), 4.40-4.49 (1H, m, Hα), 5.46 (1H, d, *J* = 7.2 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 28.4, 28.7, 36.1, 54.0, 79.5, 82.1, 155.9, 169.8, 173.2.

***O*-*tert*-Butyl-*N*-*tert*-butoxycarbonyl-L-homoserine (7).** To a solution of compound **6** (12.4 g, 42.8 mmol) in THF (300 mL) were added ethyl chloroformate (5.1 mL, 47.1 mmol) and TEA (6.6 mL, 47.1mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h, and then $NaBH_4$ (2.3 g, 60.4 mmol) was added portionwise. The reaction mixture was stirred for 12 h, and the

solvent was evaporated *in vacuo*, and the crude was dissolved in AcOEt (250 mL). The organic layer was washed successively with a 10% citric acid solution (300 mL), a sat. $NaHCO_3$ aq. (300 mL) and brine (200 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated to afford a colourless oil **7** (9.5 g, 84%); ^{1}H NMR ($CDCl_3$) δ 1.41 (9H, s, *t*-Bu), 1.43 (9H, s, *t*-Bu), 1.51-1.59 (1H, m, Hβ-a), 2.04-2.18 (1H, m, Hβ-b), 3.60-3.61-3.81 (2H, m, Hγ), 4.29-4.38 (1H, m, Hα), 5.30-5.38 (1H, m, NH); ^{13}C NMR ($CDCl_3$) δ 27.9, 28.3, 36.4, 52.3, 58.7, 80.3, 82.3, 156.6, 172.0.

***tert*-Butyl *N*-*tert*-butoxycarbonyl-(*S*)-2-amino-4-iodobutanoate (8).** To a solution of **7** (6.06 g, 22.0 mmol) in DCE (100 mL) were added DMAP (0.26 g, 2.20 mmol), Et_3N (15.3 mL, 110 mmol), TsCl (8.41 g, 44.0 mmol) at 0 ºC, followed by stirring at room temperature for 4 h. The reaction mixture was evaporated *in vacuo*, the residue was dissolved in AcOEt (200 mL), washed with 1 M HCl aq. (200 mL × 3), followed by washing with a sat. $NaHCO_3$ aq. (200 mL × 2), and finally with brine. The organic layer was dried with $MgSO_4$ and concentrated to afford a yellow oil as a residue. Without any purification, a portion of the residue was used. To a solution of the residue in acetone (50 mL) was added NaI (9.93 g, 66.0 mmol). The reaction mixture was stirred with light interception for 24 h, and poured into coled water (100 mL), followed by extracted with AcOEt (100 mL). The organic layer was washed with brine, dried with $MgSO_4$, and concentrated *in vacuo* to afford a yellow oil **8** (7.74 g, 91%); ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s, *t*-Bu), 1.49 (9H, s, *t*-Bu), 2.10-2.18 (1H, m, Hβ-a), 2.32-2.46 (1H, m, Hβ-b), 3.11-3.21 (2H, m, Hγ), 4.18 (1H, dd, J = 6.4, 11.6 Hz, Hα), 5.07 (1H, d, J = 7.2 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 14.1, 27.8, 28.3, 37.7, 60.4, 80.0, 82.5, 155.3, 170.6.

***tert*-Butyl *N*-(*p*-toluenesulfonyl)-(*S*)-2-amino-4-iodobutanoate (10).** To a solution of compound **8** (5.97 g, 15.5 mmol) in THF (50 mL) and MeOH (10 mL) was added TMSCl (10 mL). The reaction mixture was stirred at room temperature for 24 h, and then was evaporated to obtain a residue as a compound **9** quantitatively. Without any purification, compound **9** was used for next reaction. To a solution of the residue as a compound **9** in DCE (30 mL) were added TsCl (2.96 g, 15.5 mmol), TEA (6.5 mL, 46.5 mmol) and DMAP (0.19 g, 1.55 mmol). The reaction mixture was stirred at room temperature for 3 h, and concentrated *in vacuo*. The residue was dissolved in Et_2O (100 mL) and washed with a solution of 1 M HCl aq. (100 mL × 2), followed by a sat. $NaHCO_3$ aq. (100 mL), and then with brine (300 mL). The organic layer was dried with $MgSO_4$ and concentrated to afford a yellow oil (6.37 g, 94 %); ^{1}H NMR ($CDCl_3$) δ 1.26 (9H, s, *t*-Bu), 2.03-2.26 (2H, m, Hβ), 2.40 (3H, s, Ph-CH_3), 3.16-3.27 (2H, m, Hγ), 3.72-3.85 (1H, m, Hα), 5.17 (1H, d, J = 9.2 Hz, NH), 7.29 (2H, d, J = 8.0 Hz, Ph), 7.73 (2H, d, J = 8.0 Hz, Ph); ^{13}C NMR ($CDCl_3$) δ 14.1, 21.7, 28.0, 28.3, 37.7, 60.4, 80.0, 82.5, 127.4, 129.8, 136.1, 144.0, 155.3, 170.6.

***tert*-Butyl *N*-(*p*-toluenesulfonyl)-(*S*)-azetidine-2-carboxylate (11).** To a solution of **10** (3.10 g, 7.0

mmol) in MeCN (20 mL) was added Cs_2CO_3 (4.50 g, 14.0 mmol) at room temperature. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was evaporated *in vacuo*, the residue was dissolved in AcOEt (50 mL), washed with a solution of 1 M HCl aq. (50 mL × 2) and then with brine. The organic layer was dried with $MgSO_4$ and concentrated to afford a colorless solid **11** (2.30 g, quant); mp 78-82 ºC; ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s, *t*-Bu), 2.18-2.24 (1H, m, Hβ-a), 2.29-2.37 (1H, m, Hβ-b), 2.43 (3H, s, Ph-CH_3), 3.66-3.74 (1H, m, Hγ-a), 3.82-3.91 (1H, m, Hγ-b), 4.46 (1H, dd, *J* = 8.0, 9.2 Hz, Hα), 7.33 (2H, d, *J* = 8.4 Hz, Ph), 7.78 (2H, d, *J* = 8.4 Hz, Ph); ^{13}C NMR ($CDCl_3$) δ 23.2, 27.7, 44.2, 58.3, 81.0, 127.4, 129.8, 136.1, 144.0, 172.4.

***tert*-Butyl (*S*)-azetidine-2-carboxylate (12).** To a solution of compound **11** (2.4 g, 76.0 mmol) in MeOH (20 mL) was added Mg turnings (9.2 g, 382 mmol) at 0 ºC. The reaction mixture was stirred at room temperature for 2 h. After cooling the reaction mixture to 0 ºC, brine (50 mL) was added. After filtration, a solution was extracted with $CHCl_3$ (100 mL). The organic layer was dried with $MgSO_4$ and concentrated to afford a yellow oil (8.2 g, 68 %); ^{1}H NMR ($CDCl_3$) δ 1.42 (9H, s, *t*-Bu), 2.25-2.33 (1H, m, Hβ-a), 2.61-2.69 (1H, m, Hβ-b), 3.52–3.81 (2H, m, Hγ), 4.40-4.49 (1H, m, Hα), 5.22 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 23.2, 27.7, 44.2, 58.3, 81.0, 172.4.

(*S*)-Azetidine-2-carboxylic acid (2). To a solution of compound **12** (1.57 g, 10.0 mmol) in DCE (10 mL) were added water (10 mL) and TFA (10 mL) at 0 ºC. The reaction mixture was stirred at room temperature for 6 h, and then 28% NH_3 aq. was added carefully 0 ºC. After freeze-drying, compound **2** was obtained as a pale brown solid (1.01 g, quant); mp 216-218 °C (lit.,[5] mp 210 ºC); $[\alpha]_D^{20}$ -117.0 (c 0.1, H_2O) {lit.,[5] $[\alpha]_D^{24}$ -107.6 (c 3.1, H_2O)}; ^{1}H NMR (D_2O) δ 2.29-2.51 (1H, m, Hβ-a), 2.69-2.85 (1H, m, Hβ-b), 3.54–3.83 (2H, m, Hγ), 4.39-4.51 (1H, m, Hα); ^{13}C NMR (D_2O) δ 23.1, 42.1, 58.4, 172.1.

***tert*-Butyl (*S*)-1-[(*S*)-3-(*tert*-butoxycarbonylamino)-4-*tert*-butoxy-4-oxobutyl]azetidine-2-carboxylate (13).** Reactions was followed by Bouazaoui's method.[8] A solution of compound **12** (0.11 g, 0.72 mmol) in anhydrous MeCN (5 mL) was added to compound **8** (0.28 g, 0.72 mmol) dissolved in anhydrous MeCN (2 mL). The reaction mixture was stirred for 30 min under argon. Freshly distilled diisopropylethylamine (0.24 mL, 1.44 mmol) was added and the reaction mixture was diluted with anhydrous MeCN (10 mL). The reaction mixture was heated to 55 °C and stirred for 24 h. The mixture was concentrated and the crude was dissolved in AcOEt (10 mL). The organic layer was washed with 1M HCl aq., brine and distilled water. The organic phase was dried with $MgSO_4$, filtered and concentrated to afford a yellow oil. The target compound was purified by chromatography on silica gel (hexane/AcOEt, 4:1 to 1:1) to afford a yellow oil (0.18 g, 60%); ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s, *t*-Bu), 1.47 (18H, s, *t*-Bu × 2), 1.72-1.89 (2H, m, H-2), 2.04-2.28 (2H, m, H-1), 2.42-2.85 (2H, m, Hβ), 3.31-3.35 (2H, m, H-3), 3.52–3.83 (2H, m, Hα, and Hγ); ^{13}C NMR ($CDCl_3$) δ 21.3, 28.0, 28.1, 28.3, 31.1, 50.8, 53.3, 55.2, 62.0,

65.2, 80.0, 80.3, 172.1, 174.2

***tert*-Butyl (*S*)-1-[(*S*)-3-amino-4-*tert*-butoxy-4-oxobutyl]azetidine-2-carboxylate (14).** To a solution of compound **13** (67.6 mg, 0.16 mmol) in a mixture of THF (1 mL) and MeOH (0.2 mL) was added TMSCl (0.2 mL) at 0 °C. The reaction mixture was stirred for 24 h, filtered through Celite and concentrated to afford a pale yellow oil (50.3 mg, quant); ^{1}H NMR ($CDCl_3$) δ 1.43-1.55 (1H, m), 1.45 (9H, s, *t*-Bu), 1.47 (9H, s, *t*-Bu), 1.72-2.08 (2H, m, H-2), 2.29-2.52 (2H, m, H-1), 2.74-2.79 (2H, m, Hβ), 3.28-3.37 (1H, m, H-3), 3.44-3.53 (3H, m, Hα and Hγ); ^{13}C NMR ($CDCl_3$) δ 21.0, 28.0, 28.3, 31.1, 50.4, 53.1, 55.0, 65.2, 80.0, 80.3, 170.3, 174.0.

***tert*-Butyl**

(*S*)-1-[(*S*)-3-{*N*-[(*S*)-3-(*tert*-butoxycarbonylamino)-4-oxohexyl]-2-*tert*-butoxycarbonylamino}-4-*tert*-butoxy-4-oxobutyl]azetidine-2-carboxylate (15). To a solution of **14** (50.3 mg, 0.16 mmol) in MeCN (1 mL) was added Cs_2CO_3 (208 mg, 0.64 mmol) at room temperature, followed by stirring at room temperature for 30 min. A solution of **8** (52.9 mg, 0.19 mmol) in MeCN (1 mL) was added into the reaction mixture, followed by heating at 55 °C for 1 h. The reaction mixture was stirred for 24 h at room temperature, concentrated and then dissolved in AcOEt (20 mL). The organic layer was washed with 1M HCl aq. and brine, then dried with $MgSO_4$ and concentrated. Compound **15** was obtained after silica gel chromatography (hexane/AcOEt, 2:1 to 1:1) as a yellow oil (40.0 mg, 42%); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 18H, s, *t*-Bu × 2), 1.47 (9H, s, *t*-Bu), 1.51 (s, 9H, *t*-Bu), 1.56-1.80 (2H, m, H-2), 1.92-2.40 (2H, m, H-1 and H-2'), 2.61-2.95 (3H, m, Hβ and H-1'), 3.50-3.84 (4H, m, Hα, Hγ and H-3), 4.01-4.19 (1H, m, H-3'); ^{13}C NMR ($CDCl_3$): δ 16.3, 17.8, 18.0, 18.3, 21.0, 26.9, 42.5, 44.0, 54.4, 57.9, 65.6, 170.6, 171.4, 173.0.

Nicotianamine (1). To a solution of **15** (195 mg, 0.34 mmol) in a mixture of DCE (10 mL) and H_2O (0.1 mL) was added TFA (1 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h. DCE was evaporated and the resulting mixture was neutralized by the addition of 28% NH_3 aq. The crude was dissolved in distilled water (3 mL) and the aqueous layer washed with Et_2O (3 mL × 3). After freeze-drying, compound **1** was obtained as a white solid (103 mg, quant); mp 220-222 °C (lit.,[8] mp 218-220 °C); $[\alpha]_D^{20}$ -56.0 (c 0.1, H_2O) {lit.,[8] $[\alpha]_D^{24}$ -55.6 (c 0.81, H_2O)}; ^{1}H NMR (D_2O) δ 1.93-2.03 (2H, m, H-2), 2.05-2.13 (2H, m, H-2'), 2.28-2.33 (2H, m, H-1 and H-1'), 2.62-2.69 (2H, m, Hβ), 3.63-3.89 (4H, m, Hγ, H-3 and H-3'), 4.01-4.10 (1H, m, Hα); ^{13}C NMR (D_2O) δ 16.3, 17.8, 21.0, 26.9, 42.5, 44.0, 54.4, 57.9, 65.6, 170.6, 171.4, 173.0.

FITC-labeled Nicotianamine (FITC-1). To a solution of **15** (140 mg, 0.18 mmol) in a mixture of THF (10 mL) was added fluorescein 5-isothiocyanate (70 mg, 0.18 mmol) at 0 °C, and the mixture was stirred at room temperature. After 3 h, water was added to the reaction mixture, and then solvent was evaporated. The crude was dissolved in distilled DCE (3 mL), followed by the addition of H_2O (1 mL) and TFA (1

mL). The solution was evaporated again and the remaining was neutralized by addition of TEA. The crude was dissolved in distilled water (3 mL) and the aqueous layer washed with Et_2O (3 mL × 3). Compound **FITC-1** was obtained after silica gel chromatography ($CHCl_3$/MeOH, 4:1) as a yellow oil (49 mg, 98%); ^{1}H NMR (DMSO) δ 1.95–2.28 (4H, m, H-2 and H-2'), 2.45-2.79 (6H, m, Hβ and H-1 and H-1'), 3.63-3.99 (4H, m, Hγ, H-3 and H-3'), 4.17-4.25 (1H, m, Hα), 6.58-6.72 (6H, m, fluorescein), 7.29 (1H, d, *J* = 1.0 Hz, fluorescein), 7.80 (1H, d, *J* = 1.0 Hz, fluorescein), 8.00 (1H, s, fluorescein); ^{13}C NMR (DMSO) δ 16.3, 17.8, 21.0, 26.9, 42.5, 44.0, 54.4, 57.9, 65.6, 84.8, 105.7, 107.7, 109.6, 112.7, 124.0, 126.1, 129.0, 130.0, 135.5, 151.9, 159.6, 168.7, 170.6, 171.4, 173.0.

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