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DETERMINATION OF THE STRUCTURE AND ITS ABSOLUTE CONFIGURATION OF 2”-HYDROXYNICOTIANAMINE, AN INHIBITOR AGAINST ANGIOTENSIN-I CONVERTING ENZYME IN BUCKWHEAT, THROUGH THE TOTAL SYNTHESIS

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Abstract – Nicotianamine is known as an inhibitor against Angiotensin-I Converting Enzyme (ACE). We synthesized a new nicotianamine derivative with an additional hydroxy group isolated from buckwheat (*Fagopyrum esculentum* Moench) powder and determined its regio and stereochemistry unambiguously by the enantioselective synthesis of diastereomers.

INTRODUCTION

In 2006, Aoyagi (one of the authors) reported the isolation of a compound with ACE inhibitory activity from buckwheat (*Fagopyrum esculentum* Moench) powder.[1] The compound was supposed to be hydroxylated nicotianamine from its analytical data and its proposed structure is shown in Figure 1. Although its MS spectral data suggested the position of hydroxy group to be C-2” as depicted below, it has not been completely clear and its absolute stereochemistry has also been unidentified yet.

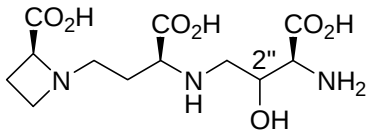
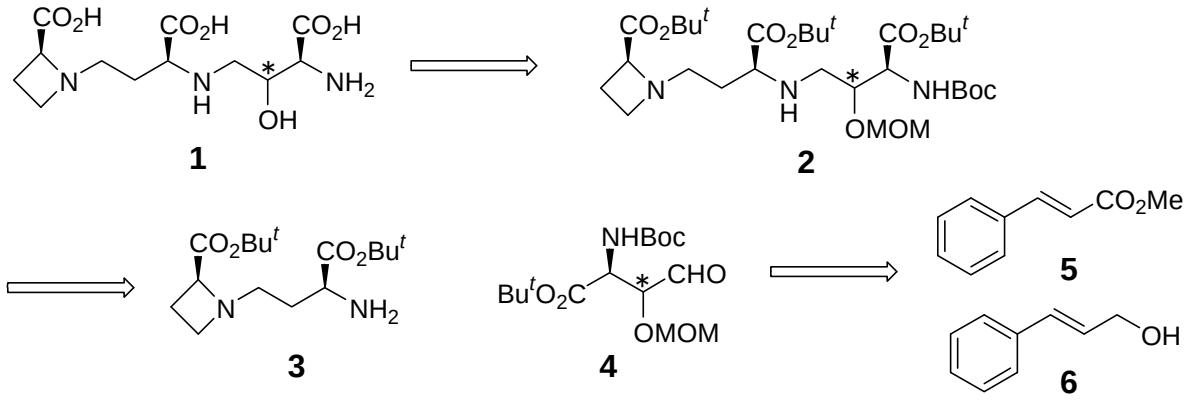


Figure 1. Proposed structure of the compound identified from buckwheat powder

During the course of our continuing study on phytosiderophores,[2] we have been interested in structure-activity relationship on nicotianamine and related analogs. Therefore, we tried to synthesize both diastereomers of the proposed structure not only to confirm the position of the hydroxy group of this compound including the stereochemistry but also to study biological activities of this natural product and congeners.

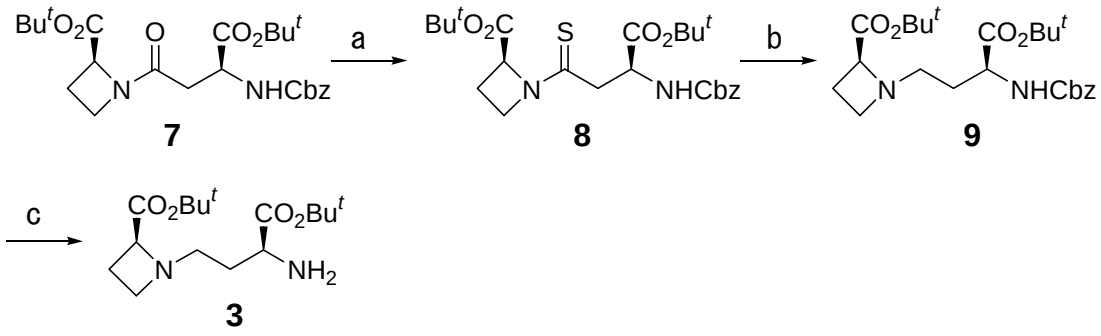
Our synthetic plan is shown below. The protected product **2** could be obtained from amine **3** and aldehyde **4** via reductive amination. Preparation of amine unit **3** must be obtainable according to the procedure previously reported by us.[2a] Both diastereomers of aldehyde **4** could be synthesized from methyl cinnamate **5** or cinnamyl alcohol **6** by Shioiri's methods.[3,4]



Scheme 1. Retro-synthetic plan of 2”-hydroxynicotianamine

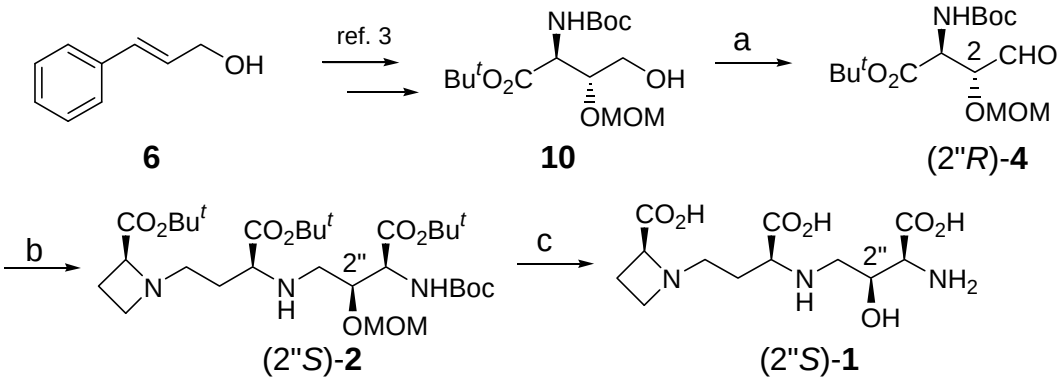
RESULTS AND DISCUSSION

The amine unit (**3)** was synthesized as shown in Scheme 2. We have already reported a preparation of the similar unit with different protective groups in 1998.[2a] In the present work, protective group of the amine moiety was replaced with Cbz and the series of reactions were performed smoothly to give amine unit **3** in a similar manner as before.



Scheme 2. Reagents and conditions: a) Lawesson's reagent, toluene, 90 °C, 93.7%; b) Raney-Ni, THF, rt, 75.8%; c) Pd-C, H_2, EtOAc, rt, 89.2%.

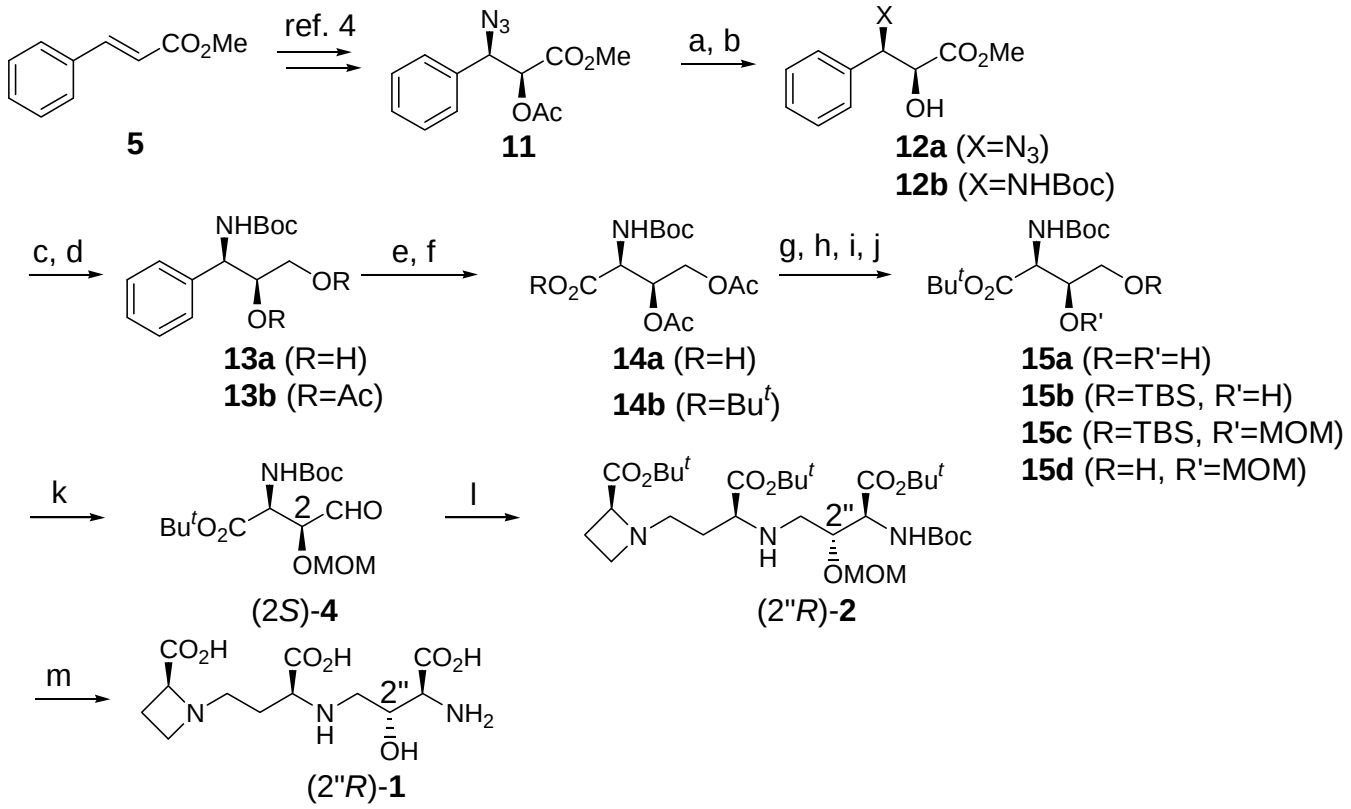
Synthesis of (2"*S*)-**1** was carried out as shown in Scheme 3. The key intermediate **10** was prepared from cinnamyl alcohol **6** by using Shioiri's method.[3] Swern oxidation of the alcohol **10** afforded crude aldehyde (2*R*)-**4**, which was submitted to the reductive amination with amine unit **3** to give the product with whole assembly. Finally, treatment with TFA, followed by Dowex purification and recrystallization from EtOH-H_2O gave pure (2"*S*)-hydroxynicotianamine [(2"*S*)-**1**].



Scheme 3. Reagents and conditions: a) $(COCl)_2$, DMSO, CH_2Cl_2 then Et_3N, −78 °C to rt; b) **3**, $NaBH_3CN$, AcOH, THF, 0 °C, 2 steps, 37.6%; c) TFA, 0 °C; Dowex 50W-X8, 30.7%.

Scheme 4 summarizes the synthesis of (2"*R*)-isomer from methyl cinnamate **5**. The known azidoester[4] (**11)** was treated with methanol and potassium bicarbonate to give alcohol **12a.** After the reduction-protection of the azide group by hydrogenolysis in the presence of Boc_2O, the product (**12b**) was reduced with lithium aluminum hydride to furnish corresponding diol **13a**. Treatment of diol **13a** with acetic anhydride gave diacetate **13b**, which was oxidized using sodium periodate and catalytic amount of ruthenium trichloride to afford carboxylic acid **14a**. The acid was converted to ester **14b** by treatment with *t*-butyl alcohol and *O*-*tert*-butyl *N*,*N*′-diisopropyl isourea (BDIU), and successive

deacetylation was achieved with triethylamine and aqueous methanol to give diol **15a**. Its primary and secondary hydroxy groups were successively protected using *tert*-buthyldimethylsilyl chloride and chloromethyl methyl ether, respectively. The silyl protecting group was selectively removed with tetra-*n*-butylammonium fluoride to give alcohol **15d**. Swern oxidation of the primary hydroxy group afforded crude aldehyde (2*R*)-**4**, which was submitted to reductive amination with the amine unit **3** in the same manner as described above. Finally, treatment with TFA followed by Dowex purification and recrystallization from EtOH-H_2O gave (2"*R*)-hydroxynicotianamine [(2"*R*)-**1**].



Scheme 4. Reagents and conditions: a) K_2CO_3, MeOH, 0 °C, 89.2%; b) Boc_2O, Pd-C, H_2, EtOAc, rt, 93.6%; c) LAH, Et_2O, rt, 69.4%; d) Ac_2O, pyridine, CH_2Cl_2, rt, 95.4%; e) $RuCl_3$, $NaIO_4$, EtOAc, MeCN, H_2O, rt; f) BDIU, Bu^tOH, CH_2Cl_2, 50 °C, 2 steps, 65.3%; g) Et_3N, MeOH, H_2O, 0 °C, 70.4%; h) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt; i) MOMCl, Pr^i_2NEt, CH_2Cl_2, reflux, 2 steps, 92.2%; j) TBAF, THF, 0 °C, 57.6%; k) $(COCl)_2$, DMSO, CH_2Cl_2 then Et_3N, –78 °C to rt; l) **3**, $NaBH_3CN$, AcOH, THF, 0 °C, 2 steps, 56.4%; m) TFA, 0 °C; Dowex 50W-X8, 54.3%.

With two isomeric **1** in hand, we compared the ^1H NMR spectrum data with that of the natural **1**, and found that (2"*S*)-**1** was identical with the natural product. In addition to that, specific rotation of (2"*S*)-**1** showed almost the same value as that of the natural **1**. [synthetic: $[\alpha]_D^{24}$ –46.6 (*c* 0.51, H_2O), natural: $[\alpha]_D^{24}$ –40 (*c* 0.4, H_2O)].

In conclusion, we have succeeded in the enantioselective synthesis of both the diastereomers of 2”-hydroxynicotianamine and by the ^{1}H NMR comparison, it was proved that the hydroxy group of the natural hydroxynicotianamine isolated from buckwheat is located at 2”-position and its absolute configuration is *S*. ACE inhibitory activities of hydroxynicotianamines and related analogs have been currently investigated and detailed results will be reported in due course.

EXPERIMENTAL

GENERAL

Melting points were measured with a BUCHI B-545. ^{1}H NMR spectra were recorded by JEOL JNM-AL300 spectrometer (300 MHz) and by a JEOL JNM-LA400 spectrometer (400 MHz), the peak for chloroform (at δ 7.24) being used as the internal standard. MS spectra were obtained with a JEOL JMS-T100LC AccuTOF, and optical rotation values were measured with a JASCO polarimeter P-1030. Column chromatography was carried out using MERCK Kieselgel 60 Art 7734.

***tert*-Butyl (2*S*,3’*S*)-1-(3’-benzyloxycarbonylamino-4’-*tert*-butoxy-4’-oxobutanethioyl)azetidine-2-carboxylate (8)** To a solution of **7** (3.95 g, 8.5 mmol) in toluene (100 mL) was added Lawesson’s reagent (1.90 g, 4.7 mmol) and the mixture was stirred at 90 °C for 2 h. The reaction mixture was filtered through celite and the filtrate was purified by chromatography on silica gel (CH_2Cl_2/EtOAc=10/1) to give **8** (3.83 g, 93.7%) as a colorless and amorphous solid; $[\alpha]_D^{20}$ –21.8 (*c* 0.35, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.45 (9H, S), 1.58 (9H, s), 2.10-2.20 (1H, m), 2.45-2.55 (1H, m), 2.81-3.11 (2H, m), 4.09-4.22 (2H, m), 4.56-4.63 (1H, m), 4.73-4.79 (1H, m), 5.10 (2H, s), 6.02 (0.5H, d, *J* = 9.3 Hz), 6.32 (0.5H, d, *J* = 9.3 Hz), 7.28-7.37 (5H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{24}H_{34}N_2NaO_6S$, 501.5913; found, 501.2037.

***tert*-Butyl (2*S*,3’*S*)-1-(3’-benzyloxycarbonylamino-4’-*tert*-butoxy-4’-oxobutyl)azetidine-2-carboxylate (9)** To a suspension of Raney–Ni (W-2) (38 g) in THF (50 mL) was added **8** (3.83 g) in THF (5 mL) at 0 °C and the mixture was stirred for 10 min at rt. The reaction mixture was filtered through celite by washing with THF and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=1/1) to give **9** (2.72 g, 75.8%) as a yellow oil; $[\alpha]_D^{20}$ –44.9 (*c* 0.09, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.42 (9H, S), 1.45 (9H, s), 1.60-1.86 (2H, m), 2.02-2.39 (3H, m), 2.69-2.75 (2H, m), 3.34 (1H, t, *J* = 7.2 Hz), 3.44 (1H, t, *J* = 8.4 Hz), 4.23 (1H, dd, *J* = 2.7, 7.8 Hz), 5.09 (2H, s), 6.20 (1H, d, *J* = 7.8 Hz), 7.28-7.37 (5H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{24}H_{36}N_2NaO_6$, 471.2471; found, 471.2487.

***tert*-Butyl (2*S*,3'*S*)-1-(3'-amino-4'-*tert*-butoxy-4'-oxobutyl)azetidine-2-carboxylate (3)** The mixture of **9** (115 mg, 0.26 mmol) and 10% Pd-C (20 mg) in EtOAc (5 mL) was stirred for 2 h under hydrogen atmosphere at rt. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=1/1) to give **3** (71.9 mg, 89.2%) as a colorless oil; $[\alpha]_D^{20}$ −12.9 (*c* 0.35, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.35 (9H, s), 1.40 (9H, s), 1.39-1.47 (1H, m), 1.69 (2H, br), 1.72-1.85 (1H, m), 2.05-2.29 (2H, m), 2.42 (1H, m), 2.71 (2H, m), 3.29-3.50 (3H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{16}H_{30}N_2NaO_4$, 337.2103; found, 337.2081.

***tert*-Butyl (2*S*,3'*S*,2"*S*,3"*S*)-1-(3'-amino-(2"-methoxymethyloxy-3"-*tert*-butoxycarbonylamino-4"-*tert*-butoxy-4"-oxobutyl)-4'-*tert*-butoxy-4'-oxobutyl)azetidine-2-carboxylate [(2"*S*)-2]** To a cooled solution of $(COCl)_2$ (466 mg, 3.67 mmol) in CH_2Cl_2 (30 mL) was added DMSO (382 mg, 4.90 mmol) at –78 °C. After the mixture was stirred for 10 min, **10** (0.82 g, 2.45 mmol) and Et_3N (742 mg, 7.34 mmol) was added successively. The mixture was stirred at the same temperature for 1 h and then at the ambient temperature for 2 h. The reaction mixture was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give crude (2*R*)-**4** (1.17 g) as an oil. This was used for the next step without further purification.

To a solution of (2*R*)-**4** (crude, 1.17 g) and **3** (1.15 g, 3.67 mmol) and AcOH (147 mg, 2.45 mmol) in THF (10 mL) was added $NaBH_3CN$ (154 mg, 2.45 mmol) at 0 °C. The mixture was stirred overnight at rt and saturated aqueous $NaHCO_3$ was added. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc= 4/1) to give (2"*S*)-**2** (582 mg, 2 steps 37.6%) as a colorless oil; $[\alpha]_D^{20}$ −17.0 (*c* 0.58, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.23 (36H, s), 1.21-1.81 (5H, m), 2.03-3.13 (7H, m), 3.30-3.49 (3H, m), 3.84 (1H, br), 4.20-4.34 (1H, m), 4.58-4.79 (2H, m), 5.93 (1H, d, *J* = 8.1 Hz), 7.24 (1H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{31}H_{57}N_3NaO_{10}$, 654.7882; found, 654.3919.

(2"*S*)-Hydroxynicotianamine [(2"*S*)-1] A solution of (2"*S*)-**2** (582 mg, 0.92 mmol) in TFA (5 mL) was stirred overnight at 0 °C. The reaction mixture was concentrated and purified by ion exchange resin (Dowex 50W-X8, H_2O then 1N aq. NH_3) to give (2"*S*)-hydroxynicotianamine **[**(2"*S*)-**1]** (360 mg) as a yellow solid. The solid was recrystallized from water (7.2 g) and EtOH (trace amount) to give (90 mg, 30.7%) of the product as a colorless solid; mp 320 ºC (decomp.); $[\alpha]_D^{24}$ −46.6 (*c* 0.51, H_2O); ^{1}H NMR (400 MHz, D_2O) 2.00-2.10 (2 H, m), 2.3-2.6 (2H, m), 3.13-3.35 (4H, m), 3.71(1H, dd, *J* = 4.4, 8.4 Hz), 3.79-4.0 (2H, m), 3.86 (1H, d, *J* = 2.8 Hz), 4.28 (1H ,m), 4.63 (1H, t, *J* = 9.6 Hz); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{12}H_{21}N_3NaO_7$, 342.1277; found, 342.1283.

Methyl (2*S*,3*R*)-3-azido-2-hydroxy-3-phenylpropanoate (12a) To a solution of **11** (1.63 g, 6.2 mmol) and MeOH (50 mL) was added K_2CO_3 (trace amount) at 0 °C. The mixture was stirred for 1 h at 0 °C and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=4/1) to give **12a** (1.22 g, 89.2%) as a white solid; mp 130.3 °C; $[\alpha]_D^{20}$ +7.8 (*c* 1.09, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 3.20 (1H, d, *J* = 6.3 Hz), 3.81 (3H, s), 4.37 (1H, dd, *J* = 3.0, 6.3 HZ), 4.86 (1H, d, *J* = 3.0 Hz), 7.24-7.35 (5H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{10}H_{11}N_3NaO_3$, 244.2024; found, 244.0715.

Methyl (2*S*,3*R*)-3-*tert*-butoxycarbonylamino-2-hydroxy-3-phenylpropanoate (12b) A mixture of **12a** (3.2 g, 14.5 mmol), Boc_2O (12.6 g, 57.9 mmol) and 10% Pd-C (1.6 g) in EtOAc (50 mL) was stirred for 2 h under hydrogen atmosphere (0.15 MPa) at rt. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=1/1) to give **12b** (4.0 g, 93.6%) as a white solid; mp 131.9 °C; $[\alpha]_D^{20}$ +7.9 (*c* 0.74, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.44 (9H, s), 3.12 (1H, s), 3.82 (3H, s), 4.45 (1H, s), 5.20 (1H, d, *J* = 9.0 Hz), 5.37 (1H, d, *J* = 9.0 Hz), 7.24-7.35 (5H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{15}H_{21}NNaO_5$, 318.1317; found, 318.1309.

***tert*-Butyl (1*R*,2*S*)-2,3-dihydroxy-1-phenylpropylcarbamate (13a)** To a suspension of lithium aluminum hydride (1.60 g, 42.2 mmol) in dry Et_2O (20 mL) was added **12b** (12.5 g, 42.2 mmol) and the mixture was stirred for 2 h under argon atmosphere at rt. To the reaction mixture was added a few drops of water, the mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=1/1) to give **13a** (7.82 g, 69.4%) as a colorless oil; $[\alpha]_D^{20}$ –3.2 (*c* 0.19, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.36 (9H, s), 3.39 (2H, m), 3.65-3.82 (3H, m), 4.63 (1H, br), 5.49 (1H, br), 7.18-7.37 (5H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{14}H_{21}NNaO_4$, 290.1368; found, 290.1356.

(2*S*,3*R*)-3-*tert*-Butoxycarbonylamino-3-phenylpropane-1,2-diyl diacetate (13b) To a solution of **13a** (7.82 g, 29.3 mmol) and pyridine (9.26 g, 117 mmol) in CH_2Cl_2 (70 mL) was added Ac_2O (8.96 g, 87.9 mmol) and the mixture was stirred overnight at rt and and saturated aqueous $NaHCO_3$ was added. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=4/1) to give **13b** (9.81 g, 95.4%) as a colorless oil; $[\alpha]_D^{20}$ –9.0 (*c* 0.34, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.37 (9H, s), 2.00 (3H, s), 2.02 (3H, s), 3.92 (1H, dd, *J* = 6.3, 12.0 Hz), 4.20 (1H, dd, *J* = 4.2, 12.0 Hz), 4.95 (1H, br), 5.16 (1H, d, *J* = 9.6 Hz), 5.32 (1H, dd, *J* = 6.3, 9.6 Hz), 7.21-7.33 (5H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{18}H_{25}NNaO_6$,

374.1580; found, 374.1570.

***tert*-Butyl (2*S*,3*S*)-3,4-diacetoxy-2-*tert*-butoxycarbonylaminobutanoate (14b)** To a solution of **13b** (9.8 g, 27.9 mmol) in EtOAc (100 mL), MeCN (100 mL) and H_2O (500 mL) was added $NaIO_4$ (149.4 g, 0.7 mol) and $RuCl_3$ (0.3 g, 1.4 mmol) at 0 °C and the mixture was stirred at rt for 24 h. The reaction mixture was extracted with EtOAc. The extract was concentrated under reduced pressure to give crude **14a** (8.49 g) as a brown oil. The crude product was dissolved in CH_2Cl_2 (70 mL) and to this was added *t*-BuOH (140 mL) and BDIU (22.4 g, 112 mmol). After being stirred for 18 h at 50 °C, the reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=4/1) to give **14b** (6.84 g, 2 steps 65.3%) as a colorless oil; $[\alpha]_D^{20}$ +27.3 (*c* 1.09, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.42 (9H, s), 1.44 (9H, s), 2.02 (3H, s), 2.04 (3H, s), 4.04 (1H, dd, *J* = 7.5, 11.4 Hz), 4.27 (1H, dd, *J* = 5.4, 11.4 Hz), 4.51 (1H, dd, *J* = 2.7, 9.6 Hz), 5.15 (1H, d, *J* = 9.6 Hz), 5.57 (1H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{17}H_{29}NNaO_8$, 398.1791; found, 398.1753.

***tert*-Butyl (2*S*,3*S*)-2-*tert*-butoxycarbonylamino-3,4-dihydroxybutanoate (15a)** To a solution of **14b** (6.7 g, 17.9 mmol) in MeOH (100 mL) and H_2O (50 mL) was added Et_3N (5.4 g, 53.6 mmol) at 0 °C and the mixture was stirred for 5 h at the same temperature. The reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=1/1) to give **15a** (3.66 g, 70.4%) as a colorless oil; $[\alpha]_D^{20}$ +27.9 (*c* 0.14, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.46 (9H, s), 1.48 (9H, s), 1.84 (1H, br), 3.43-3.66 (3H, m), 4.17 (1H, m), 4.38 (1H, m), 5.39 (1H, d, *J* = 6.6 Hz); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{13}H_{25}NNaO_6$, 314.1580; found, 314.1590.

***tert*-Butyl (2*S*,3*S*)-2-*tert*-butoxycarbonylamino-4-*tert*-butyldimethylsilyloxy-3-hydroxybutanoate (15b)** To a solution of **15a** (253 mg, 0.87 mmol), Et_3N (351 mg, 26.0 mmol) and DMAP (21.2 mg, 0.17 mmol) in CH_2Cl_2 (5 mL) was added TBSCl (393 mg, 2.61 mmol) at rt and the mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=4/1) to give **15b** (365 mg) as a colorless oil. This product was contaminated with a small amount of TBSOH; $[\alpha]_D^{20}$ +2.5 (*c* 0.19, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 0.07 (6H, s), 0.89 (9H, s), 1.43 (9H, s), 1.47 (9H, s), 2.62 (1H, br), 3.52 (1H, d, *J* = 8.7 Hz), 3.67 (1H, dd, *J* = 4.5, 9.9 Hz), 4.09-4.19 (2H, m), 5.31 (1H, d, *J* = 8.7 Hz); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{19}H_{39}NNaO_6Si$, 428.2444; found, 428.2466.

***tert*-Butyl (2*S*,3*S*)-2-*tert*-butoxycarbonylamino-4-*tert*-butyldimethylsilyloxy-3-methoxymethoxy-butanoate (15c)** To a solution of **15b** (365 mg, 0.90 mmol) and Pr^i_2NEt (700 mg, 5.4 mmol) in CH_2Cl_2

(5 mL) was added MOMCl (290 mg, 3.6 mmol) at rt and the mixture was stirred overnight under reflux. The reaction mixture was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=4/1) to give **15c** (360 mg, 2 steps 92.2%) as a colorless oil; $[\alpha]_D^{20}$ –7.5 (*c* 0.53, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 0.04 (6H, s), 0.88 (9H, s), 1.43 (9H, s), 1.45 (9H, s), 3.30 (3H, s), 3.55-3.68 (2H, m), 4.09 (1H, m), 4.35 (1H, d, *J* = 9.3 Hz), 4.57-4.67 (2H, m), 5.17 (1H, d, *J* = 9.3 Hz); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{21}H_{43}NNaO_7Si$, 472.2707; found, 472.2688.

***tert*-Butyl (2*S*,3*S*)-2-*tert*-butoxycarbonylamino-4-hydroxy-3-methoxymethoxybutanoate (15d)** To a solution of **15c** (4.19 g, 9.3 mmol) in THF (30 mL) was added 1.0 M TBAF in THF (9.3 mL, 9.3 mmol) at 0 °C and the mixture was stirred for 1 h under the same condition. The reaction mixture was concentrated under reduced pressure. The residue was diluted with Et_2O, washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=4/1) to give **15d** (1.80 g, 57.6%) as a colorless oil; $[\alpha]_D^{20}$ +85.1 (*c* 0.18, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.42 (9H, s), 1.44 (9H, s), 3.30 (3H, s), 3.43 (1H, m), 3.66 (1H, m), 3.84 (1H, m), 4.05 (1H, m), 4.44 (1H, dd, *J* = 1.8, 8.7 Hz), 4.57 (2H, dd, *J* = 6.6, 11.7 Hz), 5.36 (1H, d, *J* = 8.7 Hz); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{15}H_{29}NNaO_7$, 358.1842; found, 358.1825.

***tert*-Butyl (2*S*,3'*S*,2"*R*,3"*S*)-1-(3'-amino-(2"-methoxymethyloxy-3"-*tert*-butoxycarbonylamino-4"-*tert*-butoxy-4"-oxobutyl)-4'-*tert*-butoxy-4'-oxobutyl)azetidine-2-carboxylate [(2"*R*)-2]** A solution of $(COCl)_2$ (59.1 mg, 0.47 mmol) in CH_2Cl_2 (3 mL) was cooled to –78 °C and to this was added DMSO (48 mg, 0.62 mmol). After the mixture was stirred for 10 min, **15d** (104 mg, 0.31 mmol) and Et_3N (94 mg, 0.93 mmol) was added successively. The mixture was stirred at the same temperature for 1 h and at ambient temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give (2*S*)-**4** (253 mg). This was used for the next step without further purification.

To a solution of crude (2*S*)-**4** (Crude, 253 mg), **3** (146 mg, 0.47 mmol) and AcOH (18 mg, 0.31 mmol) in THF (5 mL) was added $NaBH_3CN$ (19.6 mg, 0.31 mmol) at 0 °C. The mixture was stirred overnight at rt and saturated aqueous $NaHCO_3$ was added. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc=4/1) to give (2"*R*)-**2** (110 mg, 2 steps 56.4%) as a colorless oil; $[\alpha]_D^{20}$ –69.6 (*c* 0.52, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.42 (36H, s), 1.69-1.81 (1H, m), 2.01-2.67 (4H, m), 2.51-2.80 (5H, m), 3.08 (1H, t, *J* = 4.8 Hz), 3.28 (3H, s), 3.43 (1H, t, *J* = 7.8 Hz), 3.99 (1H, br), 4.34 (1H, d, *J* = 9.0 Hz), 4.57 (2H, br), 5.29 (1H, d, *J*=8.4 Hz), 7.51-7.68 (1H, m); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{31}H_{57}N_3NaO_{10}$,

654.3942; found, 654.3990.

(2”*R*)-Hydroxynicotianamine [(2”*R*)-1] A solution of (2”*R*)-**2** (110 mg, 0.174 mmol) in TFA (3 mL) was stirred overnight at 0 °C. The reaction mixture was concentrated and purified by ion exchange resin (Dowex 50W-X8, H_2O then 1N aq. NH_3) to give (2”*R*)-hydroxynicotianamine (2”*R*)-**1** (60.3 mg) as yellow solid. The solid was recrystallized from water (180 g) and EtOH (trace amount) to give a colorless solid (30.2 mg, 54.3%); mp: 320 ºC (decomp.); $[\alpha]_D^{24}$ −134.9 (*c* 0.10, H_2O); ^{1}H NMR (400 MHz, D_2O) 1.98-2.1 (2H, m), 2.3-2.6 (2H, m), 3.08-3.34 (4H, m), 3.65 (1H, d, *J* = 4.8 Hz), 3.73 (1H, dd, *J* = 4.0, 8.8 Hz), 3.79-3.99 (2H, m), 4.30 (1H, m), 4.63 (1H, t, *J* = 9.0 Hz); HRMS *m/z* $(M+Na)^+$: calcd. for $C_{12}H_{21}N_3NaO_7$, 342.1277; found, 342.1283.

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